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A Summary of Current Program, 7/1/62;

and Preliminary Report of Progress

for 7/1/60 to 6/30/62

NORTHERN

UTILIZATION RESEARCH AND DEVELOPMENT

DIVISION

of the

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

This progress report of U.S.D.A. and cooperative research is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

There is included under each problem area in the report a brief and very general statement on the nature of the research being conducted by the State Agricultural Experiment Stations and the professional manpower being devoted by the State stations to such research. Also included is a brief description of related work conducted by private organizations. No details on progress of State station or industry research are included except as such work is cooperative with U.S.D.A.

The summaries of progress on U.S.D.A. and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of U.S.D.A. and cooperative research issued between July 1, 1960 and June 30, 1962. Current agricultural research findings are also published in the monthly U.S.D.A. publication, Agricultural Research. This progress report was compiled in the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Peoria, Illinois.

UNITED STATES DEPARTMENT OF AGRICULTURE
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INTRODUCTION

The Northern Utilization Research and Development Division, located at Peoria, Illinois, is one of four research divisions of the Agricultural Research Service concerned with the development of basic knowledge of chemical composition and physical properties of farm commodities and with the application of this knowledge to the development of new or improved products and processing technology that will enhance utilization of these commodities. The other Utilization Research and Development Divisions are the Eastern at Philadelphia, Pennsylvania, the Southern at New Orleans, Louisiana, and the Western at Albany, California.

The need and importance of utilization research on farm commodities arise from the fact that the farmer is not organized to carry on modern scientific research to maintain old, and create new, markets for his products. The Northern Division is responsible for utilization research concerned with industrial utilization of cereal grains, soybeans and flaxseed and with food and feed uses of corn, sorghum and soybeans. Responsibility for food and feed uses of wheat is assigned to the Western Division. In the Department's program of research on replacement crops the Northern Division conducts all screening and characterization studies on uncultivated plants and their components and is responsible for more intensive utilization research on new oilseeds containing erucic acid and on new gum and pulp fiber plants. Responsibility for detailed studies of additional selected new oilseeds is divided among the other three utilization research divisions. The Northern Laboratory also maintains small programs on sugarcane and forages. The major part of the Department's utilization research on sugarcane and forages is conducted at the Southern and Western Divisions, respectively.

In this report, utilization research of the Northern Division is discussed under the 11 Area Headings shown in the Table of Contents. For each area, a description of the current research program is provided, including domestic research contracts and sponsorship of related research performed abroad under grants or contracts involving PL 480 funds. A preliminary report of progress and a list of publications is given for each area for the period July 1, 1960 through June 30, 1962. Descriptions of related research programs conducted by State Experiment Stations and by industry and other organizations are included. These descriptions are based on a study made in 1961.

The scientific research effort at the Northern Division amounts to approximately 209 professional man-years. In addition, the Division supervises domestic research contracts equivalent to 14.3 professional

man-years and sponsors research under the PL 480 program totaling about 112 professional man-years. Following are some of the recent utilization research accomplishments of the Northern Division.

"Cereal pulp" for use in paper. New wheat flour and cereal starch products have been discovered that can be economically incorporated as an integral part of paper and other pulp products. Wheat flour and starches have been converted into water-soluble chemical derivatives that can be added to slurries of paper pulp. After a simple chemical treatment, the additives are precipitated on the pulp fibers and become part of the paper sheet. No chemical used to make the cereal product costs more than 5 cents a pound. Papers have been made in laboratory experiments containing as high as 45 percent of cereal product. The papers had higher dry and wet tensile strengths than all-wood-pulp papers. No operating conditions that would conflict with commercial paper processes were necessary.

If initial findings are borne out in larger scale tests, it is expected that the cereal product could replace 10 percent of the long fiber pulp now used in newsprint; 3 percent of the coating adhesives used in coated paper; 20 percent of the pulp used in making coarse paper; and 10 percent of the pulp used in making building and insulation board. These estimates would require the use of 100 to 180 million bushels of grain for applications not using cereal products today.

"Hidden oxidation" found to be a major factor in quality of soybean oil. The most important problem of the soybean oil industry is decrease in flavor during storage or upon exposure to heat when used as a cooking oil. On exposure to air, soybean oil immediately begins to deteriorate by oxidation of the linolenic acid component. Most of the volatile flavor components of these oxidative decomposition products are removed during the deodorizing stage of the refining. However, utilization research found that about 90 percent of the total decomposition products remain in the refined oil. Taste panel studies have shown that with time these residual products undergo further changes which reduce the flavor and oxidative stability of oil that may appear to be of high quality immediately after refining. The effects of these unremoved oxidation products have been called "hidden oxidation." Methods for detecting hidden oxidation have been developed by the Department, and industry is now using them.

Valuable chemicals found in wild plants. Substantial percentages of novel components, many having significant industrial potential, have been found in a number of the 3,400 samples of uncultivated plants analyzed in the Department's screening program. Newly discovered products include vegetable oils of unique composition and carbohydrate seed mucilages. The new oils have been converted in the laboratory to plasticizers, foamed plastics, dibasic acids for polymers, and

waxlike substances. Coproduct meals of some oilseeds have been upgraded for feed use by newly-developed processes. The seed mucilages are effective as additives for increasing paper sheet strength. Development studies with these plants are in progress.

Plant samples are obtained by the Department from both domestic and foreign sources. Projects under Public Law 480 also provide foreign species. Species desirable both agronomically and industrially can lead to development of new crops offering greater latitude in farmers' choice of commodities for profitable land use.

Linseed oil emulsion paints in commercial production. Over 50 paint manufacturers are making linseed oil emulsion paints using formulations that the Department's utilization research assisted in developing. Two linseed oil companies are producing oil emulsion bases and a third company is expected to enter the market shortly. Paint companies combine the emulsion base with pigment dispersions and other necessary ingredients to make the finished paint. If the current consumer acceptance of linseed oil emulsion paints continues, the decline in paint use of linseed oil should be halted and lost markets regained.

High-amylose corn starch available in commercial quantities. The Department's cooperation with industry on breeding and processing of a series of high-amylose corns has been the key factor in the commercial production of high-amylose corn starches, tailormade especially for a variety of industrial uses. Over three million pounds of this new corn starch containing about 61 percent apparent amylose was produced commercially from the 1961 high-amylose corn crop. Production for 1962 is estimated at five million pounds. Most of this new industrial starch is used as a size in the manufacture of glass fiber products. Further opportunities lie in industrial applications such as textile size, films, fibers, and pulp and paper additives.

New polymer made from corn sugar in semi-commercial production. Three industrial companies have announced the production of a new polysaccharide gum by the bacterial fermentation of corn sugar using a process developed by the Department's utilization research. Potential market is estimated to be multimillion pounds and each pound requires two pounds of corn sugar. Known promising uses are in oil-well drilling fluids, fire-fighting solutions, cosmetics, and pharmaceuticals. Uses in foods are under study but await Food and Drug Administration approval; preliminary feeding tests indicate the gum is nontoxic.

AREA NO. 1: CEREAL STARCHES
INDUSTRIAL UTILIZATION

Problem. Starch accounts for about two-thirds the weight of all grains. Finding new, large-volume outlets for starch would, therefore, result in substantially increased consumption of cereal grains. Of the 4.5 billion pounds of cereal starch now produced, about 2.7 billion pounds is used ultimately in food products, and increases would be expected to follow population growth. However, the remaining 1.8 billion pounds find industrial outlets that offer opportunities for increases at a rate greater than that of population growth. Because starch must compete with products derived from nonagricultural sources, these opportunities can best be realized by a program of research designed both to maintain the competitive position of starch in its current uses and to develop economical new industrial uses.

New outlets for cereal starches and flours equivalent to over 200 million bushels of grain by 1975 can be envisioned if basic research and development on several types of chemical and physical modification of starch and flour now in the experimental stage or anticipated can be prosecuted to successful conclusion. About 150 million bushels could be required for new products for the pulp and paper industries and for the building and construction industries, and about 10 million bushels each for other industries such as the chemical, petroleum, mining, textile, plastics, coatings, and packaging industries. New outlets for starch that appear very promising include use of modified starches as wet-strength additives for paper, water-resistant adhesives, coatings, and foamed products, and of starch-derived pulps as an integral part of high-quality paper. In addition, if the competitive position of starch is successfully maintained through improvement by research, additional consumption would be expected by 1975 from participation in markets for 100 million bushels of grain resulting from normal growth of existing industrial outlets for starch and flour such as paper, textiles, packaging adhesives, drilling muds, and building materials.

To accelerate realization of these goals, more information is needed on the physical and chemical properties and chemical reactions of cereal starches, on economical methods for effecting desired physical and chemical modifications and on product evaluation and development. In addition, still further new markets for cereal starches should be possible from an adequate program of fundamental and exploratory research to discover new concepts, principles, and reactions leading to new processes and products for future development.

USDA PROGRAM

The Department conducts a continuing, long-range program of research involving analytical, organic and physical chemists and chemical engineers engaged in basic, applied and developmental studies on the chemistry of cereal starches and their conversion to useful industrial products.

The Federal scientific effort for research on cereal starches totals 30.8 professional man-years. Of this number 7.9 are devoted to chemical composition and physical properties and 22.9 to new starch chemical derivatives and their evaluation.

Research at Peoria, Illinois, on chemical composition and physical properties (6.1 professional man-years) involves fundamental research on reactions of starch and dextrose in nonaqueous solvents. Research contracts under this subheading (1.8 professional man-years) are in effect with the University of Arizona, Tucson, Arizona, for basic studies on the reaction of starch with mercaptans (.6 professional man-year) and with acetylene (.5 professional man-year); and with John Hopkins University, Baltimore, Maryland, for basic research on the reactions of starch in fluid dynamic media (.7 professional man-year).

Research at Peoria, Illinois, on new starch chemical derivatives and their evaluation (21.0 professional man-years) involves basic and applied studies on various types of chemical products derived from starch, dextrin and dialdehyde starch and in evaluation of these products for various industrial uses such as plastics, coatings, pulp and paper products, organic chemicals and stable viscosity agents. During the reporting period research was discontinued on methods for producing dialdehyde starch and on evaluation of dialdehyde starch as a wet-strength additive for paper and as a component of paper coatings. Research contracts under this subheading (1.9 professional man-years) are in effect with the University of Minnesota, St. Paul, Minnesota, for studies on reactions of dialdehyde starch in solution (.4 professional man-year); with Ohio State University, Columbus, Ohio, for research on synthesis of amino derivatives of starch (1.2 professional man-years); and with the State University of New York, Syracuse, New York, for evaluation of crosslinked hypochlorite-oxidized starches in papermaking (.3 professional man-year). During the reporting period contract research on the following lines of work was completed: development of an electrolytic cell for production of dialdehyde starch; methods for using dialdehyde starch in leather tanning.

The Department also sponsors research on cereal starches (29.5 professional man-years) conducted by foreign institutions under grants of PL 480 funds. Research on chemical composition and physical properties (15.0 professional man-years) involves grants to the Institute of Fibres and Forest Products, Jerusalem, Israel, for research on

hypohalite oxidation of cereal starches (3.0 professional man-years); University of Birmingham, England, for research on starch structure as revealed by interaction of starch and enzymes (4.0 professional man-years); National Institute of Hygiene, Paris, France, for research on proteolysis inhibiting effects of cereal starches and flours (3.5 professional man-years); National Institute of Agronomic Research, Paris, France, for research on changes induced in starch by gamma irradiation (2.5 professional man-years); and Scientific Institute for Chemistry and Biochemistry, Milan, Italy, for research on glucopyranose rings in starches and dextrins (3.0 professional man-years). New starch chemical derivatives and their evaluation (14.5 professional man-years) involves grants to the Arthur D. Little Research Institute, Inveresk, Scotland, for research on glucose-derived polymers (4.0 professional man-years); Hebrew University, Jerusalem, Israel, for studies of methods for preparing fluorine derivatives of starch (2.3 professional man-years); Institute of Industrial Chemistry, Bologna, Italy, for studies on fatty chemical derivatives of starch dextrins (3.2 professional man-years); and National Institute of Technology, Rio de Janeiro, Brazil, for research on phosphorus- and sulfur-containing cationic starches (5.0 professional man-years).

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 14.2 professional man-years divided among subheadings as follows: chemical composition and physical properties, 5.4 professional man-years; industrial chemical products, 1.6 professional man-years; and processing technology, .3 professional man-year.

One phase of starch research involves establishing the mechanism of attack of oxidants such as chlorine, chlorine dioxide and hypochlorite on starch at different hydrogen ion concentrations. This work contributes to the regional study of soft wheat starches, NCM-28, Marketing, Quality and Utilization of Soft Winter Wheats. Other objectives of the regional effort include soft wheat starches, air classification and utilization of starch fractions in commercial products. The USDA Soft Wheat Laboratory participates in this regional project. Several approaches to the chemical and enzymatic modification of starch are grouped under a well-coordinated regional project plan, NCT-56, Modification of Starch for Industrial Uses.

Enzyme characterization of starch structure is also under study. Starch gelatinization, structure and reactivity of starch granules, and fundamental properties of colloid systems are also being investigated.

Use of corn products coatings for control of shrink and preservation of quality in foods is under study. Another aspect of work involves

an exploratory investigation of the production of hydrophilic polymers from carbohydrates.

Processing technology research involves development of ways to extract and characterize the various components of corn.

Industry and other organizations including the state governments also conduct research to develop new or improved industrial and food products from refined cereal starches. Such research is conducted primarily by companies engaged in wet milling of corn.

The principal research activity of most of these companies is applied and developmental research on products for industrial use. Next in importance is processing research, followed by research on new food products. Basic research is conducted by nearly all companies, several of which individually support relatively large programs. Through the Corn Industries Research Foundation, the companies support an important additional amount of basic research on corn starch. Consuming industries, such as the paper, textile, adhesive, mining and petroleum industries, maintain a very substantial research program designed to improve their own products that contain existing, commercially available starches and modified starches. Since this research is not believed to be concerned with discovery and development of new or improved products by chemical or physical modification of starch, the magnitude of this research effort is not included in the estimates given below.

Corn wet-milling companies frequently cooperate in evaluation of products and processes developed by the Department. Except for such cooperative work, the exact nature of the products and processes investigated is kept confidential by the companies or patented. Estimated annual expenditures for research on cereal starches by the companies are equivalent to approximately 90 professional man-years in basic research, 70 professional man-years in applied and developmental research on food products, 174 professional man-years in applied and developmental research on industrial products, and 90 professional man-years in research on processing.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Reactions of starch and dextrin in nonaqueous solvents. The structure of isomaltol was proved to be 2-acetyl-3-hydroxyfuran. Maltol was obtained by treatment of maltose hydrate from starch with morpholine phosphate in dimethyl formamide. Isomaltol also was formed, but in lower amounts and well below the yields previously obtained from lactose. The objective of this research is to develop procedures for

making these flavoring agents from starch. They are now obtained as a byproduct of the wood distillation industry.

Imino-bis-1-deoxy-D-glucitol (disorbityl amine), which has good metal chelating properties, was prepared by hydrogenation of di-D-glucosylamine. Disorbitylamine hydrochloride has been produced in 59-percent yield (crude) by hydrogenation of the reaction product of dextrose and ammonium chloride in dry liquid ammonia. Disorbitylamine sequestered much more copper and iron than diglucosylamine or N-methylglucamine (e.g., 1.3 vs. 0.1 atom Fe^{+++}/mole at pH 7). In strongly alkaline solutions it sequesters slightly less calcium and slightly more strontium than sodium gluconate or glucoheptonate.

The good metal chelating properties of disorbitylamine, when compared to the commercial products sodium gluconate and glucoheptonate, suggest that this product merits further consideration for industrial use. Possibilities are further enhanced by opportunities for chemical modification of this amine. For example, selective esterification with fatty acids should yield a molecule combining surface activity and sequestering ability. This type of product should have many uses in the fermentation and food industries; feeding tests for toxicity have yet to be done.

2. Starch acetylene reaction. Initial contract studies at the University of Arizona showed that acetylene reacts with starch; a degree of substitution in the range of 0.3 to nearly 1 was obtained in first experiments with no appreciable formation of undesired cyclic acetal. Thirty-two vinylations of starch by reaction with acetylene were carried out according to a fractional factorial design. Statistical analysis of the data is in progress to obtain information on reaction variables.

3. Hypohalite oxidation of starches. At the Institute for Fibres and Forest Products Research, Jerusalem, Israel, kinetic studies of the mild oxidation of wheat starch with alkaline hypochlorite have been completed. The reaction is first order with respect to starch at pH 8; activation energy 21-23 kcal/mole. Neutral salts accelerate the reaction rate. Absence of chlorate indicated that the reaction proceeds according to the equation: Starch + NaOCl $\longrightarrow$ Starch-O + NaCl. The action of hypochlorite appears to be random and similar for both wheat and waxy corn starches. Parallel studies have shown that hypobromite reacts 50 times faster than hypochlorite. Procedures have been developed (1) for determining small concentrations of carbonyl groups in starch by means of the cyanohydrin reaction, and (2) for estimating the number-average degree of polymerization of amylose and amylopectin in degraded starch without recourse to fractionation. This work is providing needed basic information on a process used to produce oxidized starches commercially in the United States.

4. Starch structure. Analytical ultracentrifugation studies, in progress at the University of Birmingham, England, have shown that properties of starch when dispersed in alkaline solution are extremely responsive to changes in the conditions of dispersion. Exact control of these conditions is necessary if the components of starch are to be obtained in a relatively undamaged state. Studies of the changes that occur during the first few minutes of dispersion have resulted in development of a method for fractionating starch into amylose and amylopectin by differential sedimentation of neutralized alkaline dispersions of starch. This research is providing important new basic information on the chemical and physical properties of starch.

5. Proteolysis inhibition by starch. The National Institute of Hygiene, Paris, France, has reported that different types of starches reduce peptic proteolysis of milk to different extents. Adsorption of proteolytic products on starch may be an important contributing factor. These studies are designed to elucidate the alleged antitryptic activity of raw wheat flour.

B. New Starch Chemical Derivatives and Their Evaluation

1. Dialdehyde starch (DAS) production and evaluation. In the final work on process development, the annular cell was improved to permit a 300-percent increase in production per cell. Based on the annular cell, a multistage reactor system was developed in which both oxidation of starch and reoxidation of the oxidant are accomplished continuously with a reduction of operating manpower. Although the continuous process has not been perfected, its feasibility has been demonstrated.

In the use of DAS as a wet-strength additive for paper at the 0.5-percent level (based on dry weight of pulp) retention of DAS was about 60 percent in the presence of cationic starch as a retention aid. By recycling the white water, retention of 98 percent of the DAS was achieved. This procedure significantly improves the economics of using DAS as an additive in paper. In addition to its use for increasing wet strength of paper, DAS offers promise as a component of adhesives needed in papercoatings. Effects of pH and concentration on crosslinking of casein by DAS in aqueous borax dispersions were investigated. Varying ratios of DAS to casein gave insoluble products differing in composition. Maximum combining power was found to be 25 g. DAS per 100 g. of casein. Viscosity stability could be achieved by maintaining reaction mixtures under slightly acidic conditions. Rapid reaction and gelation occurred under alkaline conditions. Pigmented papercoatings were made from DAS-casein dispersions that had extremely high wet-rub resistance and were superior to many coatings now used commercially.

Contract research at the Armour Leather Company showed that it is possible to reuse DAS pretanning solutions after proper fortification. This finding is of basic importance to the development of an economically attractive process. Evaluation tests at the Eastern Utilization Research and Development Division showed that sole leather comparable to regular sole leather was produced by use of DAS and vegetable tanning. Ten replicate runs based on reuse of the DAS pretanning solution were completed. Although research under the contract has been terminated, the project is being kept active to cover service tests on the sole leather to be made by the Quartermaster Research and Engineering Command.

2. Chemical reactions of dialdehyde starch (DAS). Cationic DAS's have been prepared by periodic acid oxidation of commercial cationic starch or by reaction of DAS with di- and trimethylglycine hydrazides. Preliminary evaluation studies (not yet complete) show that these new products give excellent results as wet-end additives for increasing wet- and dry-strength of paper without use of a retention aid as presently required for best results with ordinary DAS. Even though cationic DAS's are more expensive than DAS, cost of using these new products in paper, especially those derived from DAS and di- and trimethylglycine hydrazides, is decreased because of elimination of the retention aid and because they are effective at low levels of application.

Depolymerization of DAS in methanol with sodium yielded a mixture of three polyfunctional aldehydes. Production in good yields by a simple reaction of these low-molecular-weight aldehydes is a promising route to materials that should have a variety of interesting industrial applications.

Use of 2,2-diallyloxypropane to speed up and drive the reaction between dialdehyde starch and allyl alcohol to completion by removing water of reaction gives a product, cured films of which are considerably more resistant to boiling water than are those obtained when 2,2-dimethoxypropane is used to remove water. The product obtained when 2,2-dimethoxypropane is employed contains methyl as well as allyl acetal groups and does not cure as fully as completely allylated dialdehyde starch. Films cured with a free radical catalyst at 80° C. were several times more resistant to alkali than those cured by the former method, i.e., at 150° C. without catalyst. Plans are being made to negotiate a research contract covering evaluation of allylated DAS films.

At the University of Minnesota, model carbohydrate compounds were prepared that yield after periodate oxidation simple low-molecular-weight analogs of DAS. These model compounds will assist in the study of chemical reactions of DAS and of changes that occur when DAS is aged or dispersed (as in paper and tannage applications).

The course of the degradation of aged, relatively insoluble dialdehyde starch by bisulfite was followed by means of the ultracentrifuge and appeared to proceed in three stages: (a) The formation of a small quantity of low-molecular-weight material, which is solubilized before swelling of the polymer is noted; (b) liberation of a fairly homogeneous major component of molecular weight of the order of 50,000; and (c) a further degradation of this component to a heterogeneous mixture of components. Studies further revealed that for intermediate levels of oxidation (29 and 43 percent) periodate acts randomly along the starch chain.

3. Allyl starches and dextrins. The major problem in utilizing allyl starch as a coating resin is development of a derivative that has desired solubility in organic solvents and that will yield films having satisfactory properties, especially in regard to curing, brittleness, and water resistance. A number of approaches have been tried unsuccessfully in an effort to achieve this goal. Some of these approaches are crosslinking of starch with epichlorohydrin before allylation; alteration of the starch chain by low level periodate oxidation; epoxidation with peracetic acid; allylation of dextrin; hypochlorite addition and dehydrohalogenation; and derivatization with unsaturated diepoxides. Study of allyl derivatives of amylose and high-amylose starch showed that the structural linearity of these products did not provide the desired improvement in properties. Polymerization of 60 to 70 percent of the allyl groups of an allyl dextrin was achieved with free radical initiation. Degradation associated with oxidative polymerization was thus avoided, but the product nevertheless had poor resistance to water and solvents.

These results, together with the excellent properties of allyl dialdehyde starch coatings, show that configurational differences in the position of the allyl groups play an important role. They have finally revealed the predominance of intramolecular polymerization as the probable reason for failure of allyl starches and dextrins to yield satisfactory films. It is now apparent that before preparation of satisfactory polymers from these materials can be achieved, methods for eliminating or controlling intramolecular polymerization must be developed. An extensive program of basic research on such subjects as selective substitution, extension or unfolding of the allyl starch or dextrin molecule, and use of blocking groups would be required before such methods can be devised.

4. Chemical products from starch and dextrin. Starch was reacted with dihydropyran, ethyl vinyl ether and fatty vinyl ethers to yield products that give dense stable foams, gels, waxlike products, and water-resistant films. Both water-soluble and organic-soluble products were obtained. Properties varied depending on the degree of substitution.

In initial studies, hydroxymethylfurfural (HMF) has been obtained from starch and glucose in substantially higher yields (over 40 percent) than has been reported in the literature. HMF can be used for making nylon. Yields of about 80 percent from starch could make the process economical. Starch and flour have been converted by a simple process involving inexpensive chemicals into liquid products that yield rigid foams by treatment with diisocyanates. The process has been successfully applied to corn, wheat, red milo, and other starches; wheat flour; and low-protein flour fractions.

In contract studies at Ohio State University, use of alkali metal derivatives of methyl α-D-glucopyranoside as intermediates for further synthetic reactions was found to be insufficiently selective for synthesis of amino sugars. Reduction of the reaction product of dialdehyde starch and phenylhydrazine gave a polymer believed to contain amino-substituted glucose units.

At the Arthur D. Little Research Institute, Inveresk, Scotland, two new types of carbohydrate-containing polymers have been synthesized. One series consists of nylon-type polymers prepared by interfacial polycondensation, and the other of polyvinyl addition polymers which carry pendant hexitol groupings attached by ester linkages. Reactants in the interfacial polycondensation technique are sugar diamines and dibasic acid chlorides, or diamines and sugar acid chlorides. Selected polymers are currently being evaluated by industrial firms in England.

In efforts to produce surfactants and define the limits of dextrin molecular weight relative to the degree of substitution and chain-length of fatty radicals for optimum surface activity, progress has been made in fractionating a commercial corn dextrin. However, the desired low degree of fatty substitution of the dextrin fractions has not been achieved. Esterification methods are being modified to lower the degree of substitution. This research is being conducted at the Institute of Industrial Chemistry, Bologna, Italy.

5. Crosslinked starch in paper. In contract work at the State University of New York, investigations were completed on the use of crosslinked, hypochlorite-oxidized starches for beater additives; no apparent advantages over commercial oxidized starch were found for bleached pulps. Data indicated that crosslinked, hypochlorite-oxidized starch merited further consideration as a retention aid and for use in surface coatings for paper, and that further studies should be conducted with unbleached pulps.

PUBLICATIONS AND PATENTS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Chemical Composition and Physical Properties

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AREA NO. 2: WHEAT
INDUSTRIAL UTILIZATION

Problem. Wheat traditionally commands a higher price than corn. Since the starch content and starch properties of these two cereals are similar, new industrial uses for wheat must rely on advantages to be obtained from other components. Wheat flour is a mixture of starch, protein, gums, fiber, and fat. Because of the simultaneous presence of these basic ingredients, opportunities are promising for development of a wide variety of industrial products from wheat flour that would be expected to have properties and uses different from those of related products derived from refined starch. The problem is to find means for economical modification and reaction of these ingredients with each other and with other chemicals in order to realize the potential of the combinations.

Basic research now being conducted by the Department points to new potential industrial uses for cereal starches and flours that could consume over 200 million bushels of grain by 1975. Among potential outlets for wheat flour are sizes for many special grades of paper, cereal pulps that would form an integral part of such papers, and plastic or foamed compositions for hardboard and insulating boards. The opportunity for successful realization of these possibilities is enhanced by recently developed fine-grinding and air-classification milling techniques that permit the composition of flour to be varied over wide ranges. These techniques are now satisfactory for soft wheats, but ways must be found to adapt them to hard wheats which constitute 93 percent of the wheat remaining after current needs have been met.

Wheat flour could achieve its share of potential new markets more rapidly, and discovery of additional new uses under both public and private research programs would be facilitated, if more information were available on the basic physical properties and chemical reactions of flour and its components, on tempering and milling techniques, and on processing methods for economical conversion of flour to desired end products.

USDA PROGRAM

The Department conducts a continuing long-range program of research involving analytical, organic and physical chemists, chemical engineers and structural biologists engaged in basic studies of the chemical and physical properties of wheat, flour, flour fractions, and protein components and in applied research leading to new and improved wheat products for industrial use.

The Federal scientific effort for research on industrial utilization of wheat totals 45.2 professional man-years. Of this number 14.8 are devoted to chemical composition and physical properties; 18.7 to industrial chemical products; 6.8 to processing technology; and 4.9 to wheat structural products.

Research at Peoria, Illinois, on chemical composition and physical properties (12.4 professional man-years) includes separation, characterization and chemical reactions of the component proteins of wheat gluten. Research contracts (2.4 professional man-years) are in effect at Purdue University, Lafayette, Indiana, for fundamental studies of the alkaline desulfurization of gluten (.8 professional man-year); and Armour Research Foundation, Chicago, Illinois, for investigation of methods for controlled hydrolysis of gluten (1.6 professional man-years). During the reporting period contract research on isolation and characterization of sterols from wheat was completed.

Investigations on industrial chemical products conducted at Peoria, Illinois, (16.5 professional man-years) involve preparation and evaluation of new types of water-soluble and water-insoluble flour derivatives for industrial use. During the reporting period small-scale laboratory research on acid-modified flour was discontinued and pilot-plant studies on this product were initiated. Research contracts (2.2 professional man-years) are in effect with Stanford Research Institute, Menlo Park, California, for basic research on graft copolymers from wheat flour and starch (1.4 professional man-years); and Iowa State University, Ames, Iowa, for studies on development of improved adhesives from wheat gluten by reaction with dialdehyde starch (.8 professional man-year). During the reporting period contract research on evaluation of hydroxyethylated wheat flour in paper was completed.

Processing technology research at Peoria, Illinois, (6.2 professional man-years) involves studies on conditioning and milling of wheat and air classification of flours. A research contract (.6 professional man-year) with Kansas State University, Manhattan, Kansas, is concerned with study of the mechanism of enzyme formation during wheat malting and relationship of the information developed to control of enzymes and their action during milling and processing of wheat. During the reporting period contract research was completed on the effects of chemicals, such as gibberellin, on the enzyme activity of malted wheat.

Research at Peoria, Illinois, on wheat structural products (4.9 professional man-years) involves development of structural boards and foamed products from chemically modified wheat components. During the reporting period investigations on puffed-wheat particle board and on structural foams from chemically unmodified gluten and flour were completed.

The Department also sponsors research in this area (5.4 professional man-years) conducted by foreign institutions under grants of PL 480 funds. Research on chemical composition and physical properties (1.2 professional man-years) involves a grant to the Weizmann Institute of Science, Rehovot, Israel, for synthesis and study of polypeptides having amino acid compositions related to wheat gliadin. Research on processing technology (3.3 professional man-years) involves a grant to the Research Association of British Flour Millers, St. Albans, England, for investigations on quantitative measurement of properties of wheat that change significantly during conditioning.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 5.3 professional man-years divided among subheadings as follows: chemical composition and physical properties, 3.1; processing technology, 1.8; and wheat structural products, .4.

Work is in progress on the biochemical properties of cereal grains which affect their processing and use. Another research program involves determining the size, shape and structure of soft wheat proteins. The submicroscopic structure of starch gels and the influence of thermal factors and hydrogen-ion factors during retrogradation is being investigated. Enzymology of wheat as related to germ damage in storage; unidentified factors in wheat germ oil; biochemical and physical processes occurring in stored grains; and physical quality of wheat in relation to milling quality and wheat quality are other problems receiving attention. A cooperative investigation with USDA attempts to improve the interaction of wheat gluten with dialdehyde starch.

Several aspects of processing technology are being investigated. One study explores the effect of chlorine on starch in the bleaching of wheat flour. The mode of action of chlorine on starch is receiving special attention. Factors influencing wheat conditioning and the effects of high voltage cathode ray irradiation on the chemical properties and biological value of wheat proteins are other processing techniques being investigated. Efforts continue. Cooperative research with USDA involves study of enzyme formation during wheat malting.

The use of wheat and wheat products as components of structural products is also under study. Advantage is taken of certain of the chemical and physical properties of wheat and wheat fractions.

Industry and other organizations including one state government conduct valuable research on utilization of wheat. The major research interest of wheat millers and processors is directed towards food uses of wheat. Probably less than 15 percent of their total research effort pertains

to development of new industrial uses. In this area, only a small amount of basic research is in progress, with the larger share of the effort being devoted to applied and developmental research on processes and products derived from wheat flour fractions, wheat starch and wheat gluten. Some cooperative work is performed in evaluation of processes and products developed by the Department. Except for such cooperative work, the exact nature of the products and processes investigated and the results are kept confidential by the companies or patented. Estimated annual expenditures by industry for research on industrial products from wheat are equivalent to approximately 5.0 professional man-years in basic research and 31.0 professional man-years in applied and developmental research on products and processes.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Characterization of wheat gluten proteins. Basic studies on the protein components of gluten as revealed by starch gel electrophoresis in the presence of a high concentration of urea have resulted in the following revision of our nomenclature for gluten proteins:

Previous Designation	New Designation
Alcohol-insoluble fraction of gluten	Glutenin
Alcohol-soluble fraction of gluten	Gliadin
Individual components:	
α_1-gluten	Glutenin
α_2-gluten	α_1-gliadin
	α_2-gliadin
β-gluten	β_1-, β_2-, β_3- and β_4-gliadin
γ-gluten	γ-gliadin
ω-gluten	ω-gliadin

These distinctions derive from observations that starch gel electrophoresis in the presence of concentrated urea resolved gliadin into eight components, whereas glutenin did not migrate. Sedimentation showed that glutenin was a mixture of many components with molecular weights ranging from about 50,000 to many millions. The weight-average molecular weight was 2 to 3 million. Cleavage of the disulfide linkages of glutenin gave uniform protein molecules having a molecular weight of 21,000. This observation shows that glutenin is composed of polymers of the small protein molecules held together by sulfur bonds. Gliadin components have molecular weights in the 40,000 range. Reduction caused no increase in components, suggesting that disulfide bonds in gliadin are mainly intramolecular.

Evidence was obtained that intermolecular association of wheat gluten through hydrogen bonding depends on the conformation imparted to the molecules by disulfide bonding. Thus, glutenin after reduction to cleave disulfide bonds showed a greater tendency to hydrogen bond than did native glutenin. Main sites for hydrogen bonding appear to involve primary amide groups. As 10 to 98 percent of the carboxyl groups in polyglutamic acid were converted to amide groups, solubility decreased in nonpolar solvents. The material finally dissolved only in solvents that break hydrogen bonds. An almost completely amidated polypeptide showed high crystallinity and β-conformation (X-ray patterns). When the polypeptide contained only 37 percent of primary amide groups (a value similar to that for gluten) the molecule could still assume an α structure. The information obtained contributes to our understanding of the behavior of wheat gluten and is basically important to physical and chemical modification of this material.

Optical rotatory dispersion data is in general agreement with our concept that glutenin is an expandable, randomly coiled polymer. However, there is definitely some oriented structure present that may be important to its properties. It may be possible to control association of protein molecules by altering the proportion of amide groups, and to control conformation by appropriate changes in disulfide linkages and pH. Reduction of all disulfide bonds in wheat gluten would produce a material much more nearly homogeneous in molecular size and structure and therefore more suitable for chemical modification.

2. Chemical reactions of wheat gluten. Deamidation of gluten in benzyl alcohol gave a partially benzylated protein yielding films having improved wet strength and water resistance. In contrast, films from a variety of native glutens had low wet strength and were brittle even after addition of plasticizers and crosslinking agents. The properties of benzylated gluten appear to be consistent with those of synthetic polybenzyl glutamate which gave flexible, water-resistant films.

Procedures were devised for reacting acrylonitrile with gluten proteins to quantitatively block sulfhydryl groups without affecting electrophoretic patterns. This reaction offers promise as a tool not only for studying structure but also for making useful derivatives.

Gluten derivatives having unique properties were prepared by converting the primary amide groups of the protein to methyl esters. Products had increased solubility in alcohol, and most or all of the peptide bonds were retained. The products were less soluble in the presence of salts than the intact gluten, indicating that primary amide groups are important to association of gluten molecules in water. Dimethylsulfoxide solvation of gluten allows single-phase reactions to be carried out in the presence of salts without precipitation. This

discovery greatly facilitates study of such reactions as deamidation, deamination and alkylation.

3. Wheat sterols. Although laboratory work on this contract project at Iowa State University was completed some time ago, the final report, received during the present reporting period, revealed some additional information of interest. Wheat bran was found to contain a phenolic compound, not present in the germ, having the empirical formula $C_{16}H_{30}O$. At least five apparently distinct sterols in addition to β-sitosterol were isolated from germ and bran and bran steroid fractions. One of these sterols was characterized as a dihydro-γ-sitosterol, but the structures of the others were not completely elucidated.

4. Synthetic polypeptides related to wheat gliadin. Numerous model polymers and copolymers, both linear and branched in type, were synthesized from selected, component amino acids of gliadin. Pertinent chemical and physical properties of these model polymers were determined. The information obtained has been applied successfully in the synthesis of a limited number of new, water-soluble, multichain polymers of gluten. This research is being conducted by the Weizmann Institute of Science, Rehovot, Israel.

B. Industrial Chemical Products

1. Acid-modified flour. The operating limits for producing a wide range of viscosity grades of modified flour by treatment in the dry state with acid have been established. The process has been scaled up in the laboratory from 200-gram runs to 7-pound runs. Because of more efficient distribution and mixing in the larger runs, a reaction time of only 1 to 2 hours was required to achieve the same results as were obtained in 6 to 8 hours in small runs. Sizing quality of the acid-modified flours from the large runs was equal to that previously found for the small-scale preparations. Heating dry, neutralized, acid-modified flour for 7 hours at 100° C. was found to greatly improve its performance as a coating adhesive for paper. Coatings prepared with this product had wax pick values of 7-8, as compared to 8 for the commercial reference coating adhesive and only 2 for the unheated acid-modified flour.

Contract work at the Institute of Paper Chemistry on the use of hydroxyethylated acid-modified wheat flour in paper has been completed. Final conclusions were that acid-modified wheat flour, with or without hydroxyethylation, gave good dispersibility and paste viscosity properties. Surface-size tests showed effectiveness equivalent to the best commercial starch size in strength and optical properties of the paper.

In view of these favorable results, engineering studies were undertaken on the preparation of acid-modified (AM) and hydroxyethylated acid-modified (HEAM) flours in the pilot plant for larger scale evaluation at the Forest Products Laboratory. The pilot-plant equipment was modified to permit preparation of 30- to 40-pound batches and to obtain better control of the process. The pilot-plant production of AM and HEAM flours showed that product characteristics could be duplicated from run to run, and confirmed results from previous laboratory preparations. Results of small-scale sizing trials conducted at the Northern Division compared favorably with those for a good quality commercial hypochlorite-oxidized starch size.

2. Flour sulfates. A series of flour sulfates was prepared by treatment of flour with a novel polymeric sulfating agent (polyvinylpyridine-sulfur trioxide complex). The most distinctive characteristics of the sulfated flours are their high degree of dispersibility (82-98 percent), high viscosity (up to 4,300 cps. for 2-percent dispersions), and high clarity. Sulfated flours with low degrees of substitution (DS) adsorb to pulp fibers whereas those of high DS do not. A number of sulfated flours have been prepared for evaluation as beater additives.

3. "Cereal-pulp" products. Xanthates of starch, wheat flour, ground wheat, and wheat bran with degrees of substitution from 0.05 to 0.5 are coupled nearly quantitatively to pulp fibers in a matter of seconds when oxidants are added. Low-cost oxidants such as chlorine and nitrogen dioxide are satisfactory, and high wood pulp consistency appears to favor retention. Neutral to acidic conditions give best oxidative crosslinking. Papers were prepared containing up to 50 percent of these "cereal pulps." Improved strength properties, including 300-percent increase in permanent wet strength, were realized. A number of other types of paper products containing from 2 to 40 percent of cereal pulps have been prepared. These include liner and corrugating board for boxes, insulating and hard boards, and newsprint and grease-proof papers. Conventional equipment was used and the products compared favorably with commercial equivalents.

Cereal-pulp products look promising. Low levels of xanthation (degree of substitution of 0.1) appear sufficient, requiring about 4 percent added chemical in the xanthide. Technical-grade carbon disulfide is 5-1/4 cents per pound and sodium hydroxide is 3 to 4 cents. With starch or flour at 6 cents per pound, the cost for materials would be 6.6 cents per pound. Unbleached sulfate pulp is 6.75 to 7.0 cents per pound. How cereal pulps will fit into the 35-million-ton-per-year paper and paper products market is a question that will be answered only after much further research and development work. Many variables in the process, and the relation of these variables to product properties, need study.

4. New copolymers from wheat flour. Contract research at Stanford Research Institute has shown that a variety of graft copolymers can be prepared in yields up to 98 percent from starch and monomers such as methyl methacrylate, acrylamide, and acrylonitrile. Some of these products had sharp melting points, while others softened with heat and probably could be molded. Some were soluble in organic solvents, others in water. Certain of the soluble graft copolymers appeared to be film formers. Type of product was affected by ratio of starch to monomer, type of initiator (redox systems, ionizing radiation, peroxides, azobisnitriles, UV plus photosensitizer), solvent, nature of monomer, and physical state of the starch. A freeze-thaw treatment of starch was found to be about as effective as ceric ion for generation of free radicals. A significant concentration of free radicals was found to remain "captured" after irradiation with a cobalt-60 source. This research is directed towards the ultimate production of new polymeric products from wheat flour fractions.

5. Adhesives from gluten and dialdehyde starch (DAS). Contract research at Iowa State University involves investigation of the reaction of DAS with wheat gluten as a basis for improving adhesive properties of gluten. Initial effort has been directed towards study of dispersants for DAS and vital gluten to provide information needed for investigation of the DAS-gluten reaction in solution. Adhesives have been prepared from solutions of DAS and wheat gluten in various solvents. Combinations were found that gave bonds of good tensile strength, especially when the DAS was dispersed in sodium bisulfite solution.

C. Processing Technology

1. Conditioning and dry milling of Pacific Northwest wheats. Long patent flours prepared from Pacific Northwest hard and soft wheats were fractionated to yield a wide variety of high- and low-protein fractions. Brevor variety (soft white winter wheat) yielded the widest range of fractions--from 1.3 to 25.1 percent protein. Reprocessing low-protein fractions from these PNW wheats yielded fractions containing as little as 1.0 percent protein (35 percent of the original flour from Brevor SWW), and all of the other varieties tested (Omar WC, Burt HWW, and Rio HRW) yielded fractions containing under 3.5 percent protein. Both unmodified samples of the low-protein fractions and samples modified by treatment with acids or other methods show promise as industrial starches for use in manufacture of paper and gypsum board. Baking tests at the Western Utilization Research and Development Division showed that the value of the high-protein fractions for fortifying bread flour decreased in the order: Brevor SWW, Rio HRW, Burt HWW and Omar WC. Fractions were less suitable than original flours for cookies, but regrinding improved all flours for cakes.

2. Conditioning and dry milling of hard red winter wheat. In fine grinding and air classification of wheat flour, hard wheats do not yield fractions as low in protein as do soft wheats. Because surplus wheat production is comprised mainly of the hard wheats, methods are needed for effective separation of hard wheat flours into high-protein fractions for edible use and low-protein fractions for industrial use. Concho variety of hard red winter wheat was given a variety of pre-treatments to determine their effects on classification. Fractionation of standard milled flour from this wheat yielded a fraction of 6.6 percent protein. Conditioning the wheat with indirect heat permitted separation of a lower protein fraction: 5.9 percent from 150° F. treatment, 5.6 percent from 190°. Gluten was damaged somewhat in the 150° treatment and drastically at 190°. After repeated drying and wetting cycles, a fraction was separated at 5.9 percent protein. By repeated freezing and thawing a low of 5.1 percent protein was reached, but the flour was of reduced elasticity; also ash content of the flour was increased. The low-protein fraction from Wichita HRW wheat was reprocessed after various treatments including heat, defatting, enzymes, sulfur dioxide and ammonia. Variations were also made in the regrinding and reclassification operations such as varying moisture content and grinding intensity, and use of fluid energy mill instead of pin mill. The lowest protein fraction from any of these treatments still contained 5.3 percent protein. In fractionation of long patent flours from Comanche, Wichita and Triumph HRW Kansas wheats, Triumph responded best, yielding a fraction containing 4.3 percent protein.

Variations in conditioning treatment with HRW wheat have not proved effective in giving flours which will yield a fraction containing a maximum of 3 percent protein on air classification. It is apparent that new approaches and concepts will be needed to solve this problem. Microscopic studies on the flours show there are definite differences in starch-protein combinations in hard and soft flours. Use of the electron microscope for evaluating the effects of processing on the binding of starch and protein in wheat should greatly assist in determining whether separation of such a fraction from HRW wheat flour is inherently possible. Inability to produce a directly usable industrial starch fraction from hard wheat flours does not preclude possibilities that air classification may yield fractions having superior properties for chemical modification such as xanthation.

3. Enzymes in malted wheat. Contract work at Kansas State University on variations in amylase and protease activity of malted wheat induced by chemicals has been completed. Potassium gibberellate (GA-K) was the most effective chemical for increasing the enzyme activity of malts. Optimum concentration appeared to be 0.005 percent GA-K in the steep liquor. The treated malts had enzyme activity equivalent to control malts germinated 1-3 additional days. Studies of the effects of mixtures of GA-K with other chemicals revealed a few instances of

synergistic response, an observation of some theoretical significance. Use of the gibberellic acid treatment in wheat malting should be practical in view of the fact that such treatment is being practiced in the barley malting industry. Under a new contract at Kansas State University, investigations have been initiated on enzyme precursors and the mechanism of formation of amylase and protease during the early stages of germination of wheat. The objective is to obtain basic information for exploitation of enzyme development and action on the grain kernel during moisture conditioning of the grain for dry milling, especially by fine grinding and air classification of hard wheats, to allow better separation of protein from starch. Significant results have not yet been obtained.

4. Quantitative measurement of wheat conditioning variables. This research, which is being conducted at the Cereals Research Station, Research Association of British Flour Millers, St. Albans, England, is still in its preliminary stages. One interesting finding has, however, been reported. This is that appreciable amounts of scutellum and aleurone are concentrated in the fine fraction obtained by air classification.

D. Wheat Structural Products

1. Puffed wheat particle board. Extended studies have been conducted on experimental preparation, measurement of physical properties, and analysis of economic factors involved in the preparation of insulating and hard boards from commercially available puffed wheat particle board have been concluded. Insulation-type puffed wheat particle board of density up to 20 pounds per cubic foot has no advantage over similar products made from wood or bagasse. Adverse factors are (1) higher costs of raw material, puffing, resin binder, and manufacture into board, (2) lower strength and rupture values, (3) poor water resistance, (4) shrinkage of puffed kernels on spray application of urea-formaldehyde resin, (5) inherent weakness of puffed kernel, (6) vulnerability to microorganisms, insects, and rodents, and (7) no apparent advantage over puffed corn or sorghum. Higher density boards would require more raw material resulting in even larger raw material cost per square foot. Consideration was given to the use of puffed wheat to produce the resilient core in boxboard. Experimental attempts were made to cement puffed wheat kernels between two kraft liner boards. The wheat-cored boxboards made were not satisfactory from the standpoint of physical properties, and costs for puffed wheat alone were not favorable over the use of paper corrugating medium. It was concluded that puffed wheat does not offer much promise as a raw material for structural board or boxboard. Modification of wheat or wheat products by chemicals before incorporation into structural products may lead to desirable properties. This research has been completed.

2. Structural foams from gluten and flour. Research on structural foams from chemically unmodified gluten and flour has been completed. Results obtained in the last phases of the work showed that, although promising foams could be obtained from vital gluten, useful products did not result from flour. Use of calcium chloride to gelatinize starch in flour without devitalizing the gluten gave initially encouraging results, but this approach was dropped because the calcium chloride could not be removed readily or inexpensively from the final product. Preliminary results with xanthated flour (cereal pulp), a highly viscous material as is xanthated starch, suggest that these materials might be economical sources of foams. Considerable knowledge and technology on the foaming properties of wheat flour have been obtained. Application of this knowledge as opportunities arise in future investigations on industrial applications of wheat flour could point the way to development of new foamed products.

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AREA NO. 3: CORN, SORGHUM, AND OTHER FEED GRAINS IMPROVED INDUSTRIAL, FEED AND FOOD PRODUCTS

Problem. Abundant quantities of corn, sorghum, and other feed grains are now available beyond those amounts required to satisfy current needs. Both domestic consumption and export potential of these grains could be increased by development of new products for use by industry or of improved foods and feeds. Industrially, increased use of corn and sorghum will be mainly dependent upon increased markets for starch. However, flours derived from these grains are mixtures of starch, protein, and minor amounts of other components. Such mixtures have promise as raw materials for conversion to adhesives, water-soluble coatings, plastic materials, and related products that should have properties and uses different from related products derived from refined starch or wheat flour and that should contribute independently to increasing industrial markets. Isolated protein components of corn and sorghum flours should be suitable raw materials for production of useful resins and films. To achieve these utilization goals, more information is needed on basic physical and chemical properties and reactions of these flours, on the properties of component lipids, waxes, and proteins and their possible interactions with starch, and on the use of fine grinding and air classification and other new milling techniques for obtaining milled products having the most advantageous properties as industrial raw materials.

Because of the growing emphasis on increasing meat production, there is need for processes to obtain improved feed products such as high-protein feeds, mill feeds, feed concentrates, and feeds with high oil content. Such improvement could be achieved through research to obtain better knowledge of the biologically and nutritionally important constituents of corn, sorghum, and oats, to evaluate present, and to develop improved, milling and processing methods, and to ascertain the effects of such methods on the nutritional qualities of the products. In addition, because of the world shortage of protein in human nutrition, this research could enhance the export value of these grains by providing the necessary basis for development of high-protein and other food products that would be acceptable in foreign markets.

USDA PROGRAM

The Department has a continuing long-term program involving analytical and organic chemists, chemical engineers and structural biologists engaged in basic studies of the components of corn and sorghum and in application of the new knowledge gained to the development of improved processing technology leading to more effective utilization of these cereal grains.

The Federal scientific effort for research in this area totals 9.0 professional man-years. Of this number 3.2 are devoted to chemical composition and physical properties and 5.8 to processing technology.

Research on chemical composition and physical properties is conducted at Peoria, Illinois, and involves investigations of physiologically active nonprotein nitrogen substances in corn and of carotenoid pigments of corn, corn milling fractions, and yellow endosperm sorghum. A portion of the effort on carotenoid pigments is cooperative with Crops Research Division and is directed to development of corn and sorghum varieties having high carotenoid content. Such varieties are needed for improved food and feed products and to enhance the competitive position of U. S. corn in international trade.

During the reporting period specific studies of the pellagragenic factor of corn were deemphasized in favor of a broader and more general investigation of nonprotein nitrogen substances. Cooperative studies with Iowa State University on vitamin and amino acid content of new oat strains were discontinued. Contract research on the nature of gums occurring in oats was completed.

Processing technology research, also conducted at Peoria, Illinois, involves pilot-plant studies of conditions and methods for increasing the yield of oil and grits by dry-milling processes. Effects of processing variations on industrially and biologically important components of corn are determined.

The Department also sponsors research (7.1 professional man-years) in this area conducted under grants of PL 480 funds to the following foreign institutions: Research Association of British Flour Millers, St. Albans, England, for studies of antioxidants occurring in oats (2.0 professional man-years); National Institute of Agronomic Research, Paris, France, for basic studies of the physical chemical properties of corn zein (3.0 professional man-years); and Weizmann Institute of Science, Rehovot, Israel, for research on synthetic polypeptides with amino acid compositions related to zein (2.1 professional man-years). These lines of work are under the subheading chemical composition and physical properties.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 0.2 professional man-year devoted to chemical composition and physical properties. Emphasis is placed on extraction and characterization of corn protein.

Industry and other organizations also conduct interesting and valuable investigations in this research area. Essentially all of the industrial research on developing new and improved industrial and food products from corn and its components, exclusive of refined corn starch,

is believed to be conducted by corn dry-milling companies. Applied and processing research on feed products is largely conducted by corn wet-milling companies and to some extent by the dry millers. Food manufacturers and brewers endeavor to improve products based on existing materials available from corn to meet competitive situations or to solve other problems incidental to company operations; however, except for certain food manufacturers and brewers who also engaged in corn milling, no research to derive or develop new materials from corn for use in their products is believed to be involved. Of the dry-milling companies, less than one-fourth are large enough to maintain a significant research program. Their largest effort is directed to process and application studies for food and brewing uses of corn. Only a small amount of basic research is conducted in this area. Interest has been increasing in industrial markets for corn flour and protein, and a number of companies are conducting applied and developmental research in this field. No basic research appears to be in progress on industrial products from corn flour. Industrial research on sorghum is small and is directed towards processing for industrial uses. Several companies have cooperated in evaluation of products and processes developed by the Department. Except for cooperative work, the exact character and results of their research are kept confidential by the companies or patented. Estimated annual expenditures for research in these areas are approximately 5.0 professional man-years in basic research on food products, 45.0 professional man-years in process and application research on foods from corn, 20.0 professional man-years in applied and processing research on feeds, and 25.0 professional man-years in applied and developmental research on industrial uses of corn and sorghum.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Nonprotein nitrogen substances of corn. A new chromatographic procedure has been developed for separation and determination of low-molecular-weight nicotinic acid derivatives present in extracts of corn. The method can be used for analysis of numerous extracts of various corn grain fractions and milling products and for ascertaining the distribution of such components in grain and changes that may occur during processing.

Chromatography of the bound niacin substance on alumina columns showed that pigment is not an integral part of this substance. The molecule is, however, a complex structure containing carbohydrates, amino acids and other components as well as bound niacin.

Forty-five percent of the free amino acids of corn is in the germ. One-third of the nitrogen in steep liquor is nonprotein nitrogen, more than can be accounted for from the nonprotein nitrogen in whole grain.

Analyses were therefore performed for all of the free amino acids in whole corn; germ, endosperm and bran fractions; and corn steep liquors. Individual amino acids of acid hydrolyzates of corn steep liquors were also determined. A component chromatographically similar to betaine was observed in whole corn, endosperm and germ. Trigonelline and choline levels in corn steep liquor were higher than in the grain. The results of the analyses show that bacterial and enzymatic hydrolysis of corn protein and further modification of the liberated amino acids takes place during steeping. Commercial steeping appears to extract effectively nonprotein nitrogen components from corn.

2. Corn and sorghum carotenoids. Yellow corn and sorghum, which owe their color to carotenoid pigments (carotenes and xanthophylls), have enhanced value as feed for cattle and poultry. Thus, carotenes are the precursor of vitamin A and in cattle feeding are the source of yellow color in milk and body fat. Xanthophylls impart the yellow color to egg yolks (the basis for commercial grading) and to the skin and fat of broilers and fryers. The present project was undertaken to investigate differences in the amount and chemical identity of individual carotenoid pigments of yellow corn and milled fractions and of yellow grain sorghums in order to provide information on variations among grain varieties and milled fractions and on changes during processing that could influence the commercial value of these feedstuffs.

An improved chromatographic procedure was developed for determination of total carotenes and total xanthophylls in corn and sorghum. Statistical evaluation showed that the method would be suitable for screening purposes, even for samples available in only limited quantities. Application of the method to 110 samples of hybrid and inbred corn showed considerable variation in the range of carotenes (0-5 p.p.m.) and xanthophylls (0-45 p.p.m.). Argentine flint corn contains up to 60 p.p.m. xanthophyll compared to yellow dent hybrids with 25 p.p.m. Initial studies on carotenoids in wet- and dry-milled fractions of corn showed that variation of carotenoid content of two samples of gluten was of the same magnitude as that in the parent whole corns processed. Carotenoid content of gluten feed and meal appeared to depend on the type and amount of materials blended during processing.

Cooperative studies with Crops Research Division have correlated the parental source of the yellow genes with xanthophyll contents of first-cross progeny. Carotenes and xanthophylls appear to be independently inherited, precluding estimation of xanthophylls from carotene content. An unsupplemented poultry ration containing 60 percent corn will contain the desired level of 25-27 p.p.m. xanthophyll if the corn contains 42-45 p.p.m. or nearly twice the normal amount; the xanthophyll content of pure lines available for hybrid combination are within this range.

Grain from the best yellow endosperm sorghum examined to date contained only 5.6 p.p.m. total carotenoids as compared to yellow corn (24 p.p.m.) grown in the same area. Grain sorghum exposed to weathering after pollination retained only 50 percent of the carotenoids present in protected seed heads. The low carotenoid content of available varieties of sorghum and the adverse effects of weathering constitute major problems in the program for breeding high carotenoid sorghums.

3. Oat gums and antioxidants. Significant interpretations of previously reported data on oat gums were revealed by the final report on this contract project at the University of Minnesota. β-D-glucan, the main viscosity producing carbohydrate, was found to possess new features of chemical structure for this class of carbohydrates. Whereas most of the D-glucose units are joined by alternating β-1,4 and β-1,3 linkages, a few segments of chains contain two to four consecutive β-1,3 linkages and others two or more consecutive β-1,4 linkages. The gum-degrading enzyme preferentially attacked the consecutive β-1,3 linkages, which also appeared to be involved in the degradation that occurs during manufacture of rolled oats.

At the Cereals Research Station, Research Association of British Flour Millers, St. Albans, England, automatic oxygen absorption apparatus has been developed to permit the measurement of induction periods on as little as 0.1 g. fat. This apparatus facilitates the measurement of the effects of antioxidants upon lipid oxidation and helps clarify the quantitative relationship between oxidation and organoleptic rancidity. A major antioxidant present in raw oats has been isolated from the grain and characterized. It was shown to be an ester consisting of one mole each of ferulic and caffeic acids and 2,6-hydroxyhexacosanoic acid. These developments are significant contributions to the problem of stabilization of lipids in baked oat products for retention of flavor.

4. Synthetic polypeptides related to zein. Numerous model polymers and copolymers, both linear and branched in type, were synthesized from selected, component amino acids of zein. Pertinent chemical and physical properties of these model polymers were determined. The information gained from these studies has been applied successfully in the synthesis of a limited number of new, water-soluble, multichain, polymers of zein. This research is conducted at the Weizmann Institute of Science, Rehovot, Israel.

B. Processing Technology

1. Corn dry milling. A statistically planned series of experiments has provided data on the effects of the following variables on yield and characteristics of degerminator products: first temper moisture, tail gate loading, feed rate, size of screen perforations, throughput,

and rotor speed. This information is important to millers in showing how to achieve the most efficient use of the degerminator.

A procedure for shortening temper time was discovered. Corn was immersed in water for 1 minute under a vacuum of 5 inches of mercury and 9 minutes at atmospheric pressure and drained 5 minutes. Degermination gave increased yield of -4 + 6 grits and decreased yield of -6 + 8 grits. Both fractions contained less oil. Except that degerminator throughput was 20 percent lower, overall results were superior to, or compared favorably with, those obtained by a conventional procedure based on tempering times of 3.25 hours. However, response varied materially with the source and age of the corn. A patent has been allowed on this process and industry has expressed considerable interest.

Caustic tempering gave reduced oil content in milled products with some lots of corn but not with others. Hull release and polish were, however, improved in every instance.

Additional rest time after conditioning is needed to obtain high degerminator capacity with some varieties of corn that have been vacuum-tempered. Combined caustic and vacuum tempering improved oil recovery to the level of conventional tempering and gave better quality product.

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AREA NO. 4: HIGH-AMYLOSE CORN INDUSTRIAL UTILIZATION

Problem. Varieties of corn have been achieved genetically that contain greatly increased amounts of amylose. Amylose, the linear fraction of starch, possesses film- and fiber-forming properties not available in ordinary starch which contains only about 27 percent of this component. Because the unique properties of amylose open areas of utilization closed to ordinary starch, the potential industrial value of this new crop is very high. Several problems must be solved, however, to realize this potential.

For high-amylose starch to have substantially improved properties as a raw material in comparison with ordinary starch, it should contain at least 80 percent of amylose. Although a number of breeding samples have contained as high as 75-80 percent of amylose, only varieties containing 50-60 percent have so far been commercially available. Even at this amylose level, however, over 4 million pounds of high-amylose starch from first commercial plantings were utilized by industry in 1961. Although breeding is the task of the geneticist, utilization research is needed to provide information on amylose content, on changes in quantities and properties of the amylose, amylopectin, and other components such as oil and protein, and on milling characteristics of breeding samples in order to insure availability of satisfactory varieties.

A second problem is development of methods for economical isolation of pure amylose from high-amylose starch. For some anticipated uses, nearly pure amylose may be required for optimum properties. Although achievement of 80-percent high-amylose starch appears certain, it may not be possible to obtain much higher levels through breeding. Success in devising efficient fractionation methods will depend upon availability of adequate basic information on freeing and separating amylose from remaining starch components.

Finally, to insure utilization of the potentially large volume of high-amylose starch that could eventually become available, more information is needed on the chemical and physical properties of amylose and high-amylose starch and on methods for converting them economically to desired products. Success in this research could lead to an estimated consumption of over 600 million pounds of high-amylose starch by 1975 in films, fibers, plastics, coatings, and related products to which the linear character of amylose could make contributions.

USDA PROGRAM

The Department conducts a long-term, continuing program of research involving analytical, organic and physical chemists, structural biologists, and chemical and mechanical engineers who are engaged in basic and applied research designed to increase knowledge of the properties and reactions of amylose and other components of high-amylose corn and to utilize this knowledge in development of attractive industrial applications for amylose and high-amylose starch.

The Federal scientific effort for research on utilization of high-amylose corn totals 20.5 professional man-years. Of this number 12.4 are devoted to chemical composition and physical properties; 7.6 to industrial utilization; and .5 to processing technology.

Investigations on high-amylose corn are conducted at Peoria, Illinois. Research on chemical composition and physical properties involves study of amylose content of breeding samples, starch and starch granule composition, structure and properties; and composition and properties of proteins and other components of high-amylose corn. Research on industrial utilization is devoted to studies on separation of amylose from high-amylose starch, and formation and properties of amylose films. During the reporting period contract research on evaluation of high-amylose starch in paper was completed. Research on processing technology involves milling studies on breeding samples and histological studies on high-amylose corn. During the reporting period intensive examination of milling properties of high-amylose corn was discontinued and such work is now performed on an "as needed" basis. Studies on amylose content and milling properties of breeding samples assist geneticists in developing varieties of high-amylose corn having increased amylose content and improved milling properties. Cooperation with Field Crops Research Branch, Crops Research Division, is maintained in conducting these studies.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 2.8 professional man-years devoted to chemical composition and physical properties. Characterization and chemical determination of the amylose content of starch from newly developed lines and varieties of corn is an important aspect of station research which supports corn breeding programs. Station researchers are sampling several open-pollinated varieties of corn to determine the natural variation which occurs in amylose content.

Industry and other organizations also perform valuable research involving high-amylose corn. Because of the present experimental nature of the crops, research on uses of starch from high-amylose corn is conducted by a very small number of companies who have contracted with

farmers to have small acreages of the crop grown for their use. The exact character of the research conducted on high-amylose starch and the results are kept confidential by the companies or patented. The Corn Industries Research Foundation supports basic research on high-amylose starch at the Northern Division (approximately .7 professional man-year). The results of this research are, of course, made public. Estimated annual expenditures by industry for research on high-amylose corn starch are equivalent to approximately 4.0 professional man-years in basic research and 6.0 professional man-years in applied and developmental research.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Amylose content of breeding samples. During the reporting period 36,292 samples of high-amylose corn were analyzed. These were supplied under Memoranda of Understanding by the Bear Hybrid Corn Company, by the Missouri Agricultural Experiment Station and by Crops Research Division at the Missouri Station. Amylose content for nearly 200 of these samples was over 75 percent and the highest value was 79.3 percent. (In this report percentages of amylose refer to apparent values determined by iodine titration. True amylose content, measured by quantitative fractionation, averages about 80 percent of the apparent value.) In 1961, over 1,000 acres of class 5 (55-60 percent) high-amylose corn was planted, marking commercialization of this new crop. Class 6 (60-70 percent) high-amylose corn is reported to be ready for commercialization. Class 7 (70-80 percent) is expected to be ready for commercialization by 1965.

2. Properties of components of high-amylose starch. Fractionation of a series of high-amylose corn starches by the solvent pretreatment-alkaline extraction procedure was rechecked, useful variations of the procedure were incorporated, and original findings were confirmed: (1) Amyloses from dent and high-amylose corn starches are comparable in iodine binding power and molecular weight; (2) by iodine binding and β-amylase conversion, the amylopectins of high-amylose corn starches show increased length in the external branches when compared with the dent corn amylopectin.

In laboratory studies on fractionation of high-amylose starch by aqueous extraction-sedimentation procedures after hydration-freezing pretreatment, recent findings indicate that (a) restoration of normal moisture content after drying starch increases yield and purity of amylose; (b) corn steeped in aqueous sodium sulfide or diethyl dithiocarbamate yields amylose of high purity (90-92 percent) than corn steeped in aqueous sulfur dioxide (85-88 percent); (c) pretreating starch in an autoclave (105-120°) greatly increases rate of

liberation of amylose; and (d) for all types of starch available three extractions at 100-101° are needed to recover 93-95 percent of the amylose present.

An improved medium (2M lithium bromide plus 4M guanidinium chloride) has been found for dispersing amylose, amylopectin and other starch fractions for molecular-weight determinations. Crystallinity and density of granules of potato starch and of two high-amylose corn starches decreased as amylose content increased.

3. Proteins of high-amylose corn. Research has been undertaken to obtain information on differences among proteins of corn containing varying amounts of amylose. The first phase of the work involves efforts to characterize proteins of ordinary dent corn. The components of native and commercial zein were separated with the aid of agar- and starch-gel electrophoretic techniques. Highly significant differences in composition were demonstrated. Native zein showed three major and nine minor components. Some material remained in evidence at the origin of the electrophoretic pattern. In contrast, commercial zein showed only five components with little material at the origin. Reductive cleavage and alkylation or performic acid oxidation of the disulfide bonds of native zein gave products that behaved electrophoretically like commercial zein.

Gel electrophoretic analysis of α- and β-zein showed that α-zein contains four components whereas β-zein does not migrate. After reduction of disulfide bonds, α- and β-zein each contain four components having correspondingly similar mobilities. In addition, reduced β-zein contains two fast-moving components not present in α-zein. Native zein containing at least five electrophoretic components gave an average molecular weight of 44,000 by sedimentation. After cleaving disulfide bonds, a molecular weight of 20,000 was obtained, which agrees with the minimum molecular weight calculated from amino acid composition of whole zein. The globulin fraction of whole corn was resolved into 17 components by starch gel electrophoresis.

These observations show that the slower moving minor components of native zein are aggregates derived by disulfide bonding of the major component proteins and that, in commercial zein, sulfur dioxide used in steeping the corn has cleaved the disulfide bonds originally present.

B. Industrial Utilization

1. Fractionation of high-amylose starch. In recently undertaken engineering studies, either the freezing pretreatment or a variety of mechanical and chemical pretreatments of 67-percent high-amylose starch followed by aqueous leaching gave similar results: 53-percent

recovery of 84-86-percent pure amylose. Fractionation by complexing with capric acid gave 86-percent yields of 83-percent pure amylose.

2. Amylose films. Wet-tensile strength was increased materially when film was immersed in hot saturated ammonium sulfate. The film, in contrast to its behavior in water, remained intact at temperatures up to the boiling point of the bath (107° C.) while partial dehydration took place. Immersion at this temperature for 15-30 seconds resulted in an increase of wet-tensile strength from about 200 pounds per squre inch cross-section to 400-500 pounds. At the same time the water-binding capacity of the film was permanently reduced from approximately 75 percent to about 60 percent (based on the weight of blotted film vs. bone-dry weight). Lesser, although still useful, effects were attained at a temperature of 85° C.

Extrusions embodying the above dehydrating technique have resulted in improved operational speed. There is still need, however, for effecting dehydration more rapidly or, alternatively, for supplying interim strengthening while dehydration is proceeding.

3. High-amylose starch in paper. Contract research at the Institute of Paper Chemistry on evaluation of high-amylose starch in paper was completed. One unmodified and eight modified samples of high-amylose starch were evaluated as beater adhesives, fiber deflocculants, surface-sizing agents and pigment-coating adhesives. The most consistently effective material was a 6.47 percent hypochlorite-oxidized starch. All samples were ineffective as deflocculants. Alkali-dispersed unmodified high-amylose starch gave excellent strength properties as beater additives and surface sizes. For certain specific areas and conditions of application, high-amylose starch samples were somewhat superior to the corresponding ordinary starch product.

C. Processing Technology

1. Wet-milling high-amylose corn. During the past 3 years, processing properties of available samples of high-amylose corn were compared with those of ordinary dent hybrids. Variations in milling characteristics of different samples of high-amylose corn were found. These variations appeared to be related to the genetic history of the samples, and have led to efforts by geneticists to breed milling quality into new strains. As a result, there is promise of new strains of high-amylose corn with greatly improved milling quality. Improved millability was observed for samples of amylomaize varieties bred to contain the so-called "milling factor" (M). In the absence of M, protein content may be an influencing factor but amylose content is not.

Present analytical methods indicate that high-amylose corn contains less starch but more protein and oil than dent corn. However, discrepancies were observed that suggest that conventional analytical

methods may not be suitable for high-amylose corn. Further investigations are in progress.

High-amylose corn was found to have two detrimental milling characteristics that need to be improved by selective breeding:

(1) Starch contains a greater percentage of small starch granules than does dent corn; these small granules are lost in the washings or remain in the gluten resulting in lower starch recovery.

(2) Kernels of high-amylose corn swell from 50 to 100 percent more during steeping than do ordinary corn kernels, giving a softer steeped grain requiring larger tanks for equal production capacity.

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AREA NO. 5: WHEAT AND CORN
FERMENTATIVE CONVERSION TO NEW INDUSTRIAL, FEED AND FOOD PRODUCTS

Problem. By fermentation of cereal grain substrates, new products can be obtained that are not readily available by other means and have promising potential for industrial, agricultural, and food uses. Processes now under development, if brought to successful conclusion, could lead to increased consumption of an estimated 40 million bushels of grain for fermentative conversion to stable viscosity agents for secondary petroleum recovery by flooding of spent oil wells, to new organic acids and enzymes for industrial use, to feed supplements, and to effective biological insecticides and other pesticides that are harmless to man. In addition, there are good possibilities for utilizing fermentation processes to produce new food products that should promote foreign use of U. S. grains.

To accomplish these objectives and to realize the full potential of fermentative techniques for increasing utilization of grain, a broad program of exploratory research is required to find and identify through taxonomic studies species of organisms producing potentially valuable products, to isolate high-yielding strains or develop them by mutation, hybridization or genetic selection, and to develop basic information on culture media, special nutrients, and other factors required for optimum growth of microorganisms and maximum yields of desired products. Continued maintenance and expansion of a collection of pure cultures of well-characterized organisms is necessary for this research. For successful translation of laboratory results into commercially useful processes, more information is needed on new techniques of fermentation, on development of economical methods of growing organisms and handling fermentation processes on a large scale, and on special procedures for efficient isolation and purification of products from fermentative reaction mixtures. Finally, the most appropriate end uses for products must be identified and information obtained on product evaluation and development.

USDA PROGRAM

The Department has a long-range continuing program involving analytical and organic chemists, biochemists, microbiologists, systematic biologists, and chemical engineers engaged in basic research on microorganisms and microbiological reactions and products and in application of both known and newly discovered principles to the development of practical fermentation processes for conversion of cereal grain substrates to useful chemical and biological products.

The Federal scientific effort in this area of research totals 46.2 professional man-years. Of this number 15.3 are devoted to basic research on fermentation processes; 17.7 to industrial chemicals; 9.9 to biological pesticides; and 3.3 to feed and food products.

Basic research on fermentation processes conducted at Peoria, Illinois, (15.3 professional man-years) includes study of taxonomy of molds, yeasts and bacteria; factors affecting viability of microorganisms; and microbiological reactions and products. Basic to these investigations and to the Division's entire research program on fermentation is assembly and maintenance in pure culture of a large collection of agriculturally and industrially important microorganisms. Much of the research on microbiological reactions and products is conducted by the Pioneering Laboratory for Microbiological Chemistry. During the reporting period exploratory research on microbial hydroxylation of unsaturated fatty acids was discontinued. General studies of the taxonomy of actinomycetes and of yeasts of the genus Hansenula were completed.

Research at Peoria, Illinois, on industrial chemicals (17.7 professional man-years) involves fermentative production of microbial gums, organic acids, and other products for use in the chemical industry. During the reporting period research on six selected microbial polysaccharides was completed as was work on microbial deamidation of wheat gluten and microbial production of polyglutamic acid from gluten. Also, contract research was completed on development of methods for isolation of α-ketoglutaric acid from fermentation liquors.

Research at Peoria, Illinois, on biological pesticides (9.4 professional man-years) is devoted to studies on biological insecticides for Japanese beetle, other insect control agents and plant antibiotics. Investigations on biological insecticides for Japanese beetle and on other insect control agents is cooperative with Entomology Research Division and Plant Pest Control Division. Research on plant antibiotics involves cooperation with Crops Research Division. A research contract (.5 professional man-year) is in effect with Michigan State University, East Lansing, Michigan, for study of factors important to large-scale propagation of Japanese beetle pathogens. During the reporting period broad screening investigations of microbial plant antibiotics and specific studies of F-17 antibiotics were terminated in favor of restricted screening for antibiotics showing activity against a selected group of plant pathogens that can be cultured in the laboratory.

Research at Peoria, Illinois, on feed and food products (2.8 professional man-years) involves study of production of microbial carotenoids suitable for feed supplements. A research contract (.5 professional man-year) with Michigan State University, East Lansing, Michigan, concerns evaluation of biological availability of fermentative β-carotene when fed to poultry and swine.

The Department also sponsors research in the fermentation area (23.4 professional man-years) conducted by foreign institutions under grants of PL 480 funds. Basic research on fermentation processes (15.0 professional man-years) involves grants to the National Institute for Agronomic Research, Madrid, Spain, for collection of new species of yeast (2.0 professional man-years); University of Helsinki, Finland, for basic studies on organic phosphorus compounds of yeast (3.0 professional man-years); University of Milan, Italy, for basic studies on the metabolic pathway to 2-ketogluconic acid in Acetobacter species (2.8 professional man-years); University of Allahabad, India, for collection of new Mucorales species (2.1 professional man-years) and studies on survival of lyophilized microorganisms (2.1 professional man-years); and University of Durham, Newcastle-upon-Tyne, England, for investigations of sugar phosphate derivatives in molds (3.0 professional man-years). Research on industrial chemicals (4.4 professional man-years) involves a grant to Superior Institute of Health, Rome, Italy, for studies on foaming in anaerobic fermentations. Research on feed and food products (4.0 professional man-years) involves a grant to the "Giuliana Ronzone" Scientific Institute of Chemistry and Biochemistry, Milan, Italy, for research on production of vitamin B_{13}.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 2.2 professional man-years under the subheading biological pesticides. One program involves basic work directed toward developing information on the physiology of molds which may be of industrial importance. Another program involves characterization of amino acid addition products isolated from plant materials.

Industry and other organizations conduct a large program of research in the area of fermentation. In the field of industrial chemicals, fermentation acids are usually manufactured from cheap foreign molasses when available. However, production of some acids requires cereal carbohydrate, and all common acids can be made from this substrate. Grain products and byproducts are prevalently used for production of microbial enzymes. Research by the companies is directed primarily to process development and improvement and to evaluation and application of products. Some of the effort concerns development of processes based on basic and applied research of the Department. Relatively little basic research is performed by the companies. Isolates are maintained and taxonomic work performed only in the area of interest.

Biological pesticides and related products are made by fermentation of cereal grains and grain byproducts. Research by the companies is directed principally towards process development and improvement and testing of products. A substantial part of the effort in this field

concerns processes based on results of research conducted in the Department. A relatively small amount of basic research is conducted.

Animal feed supplements containing vitamins, antibiotics, enzymes, and other microbial products are manufactured largely by fermentation processes based on cereal grains and grain byproducts. A considerable research effort is devoted by industry to improvement of existing processes, to the development of new products and processes, and to evaluation of products. Several companies are engaged in discovery and development of microbial products containing so-called "unidentified growth factors." Some basic research is performed. Although quantities of human foods are prepared by fermentation processes, there is very little activity in regard to fermented foods derived from cereal grains.

Except for cooperative work that may result from preliminary evaluation of products and processes developed by the Department, the specific nature of the research in these fields and the results are kept confidential or patented by the companies. Estimated annual expenditures for research in these areas are equivalent to approximately 30 professional man-years in basic research, 90 professional man-years in applied and developmental research on industrial chemicals, 35 professional man-years in applied and developmental research on biological pesticides, less than 5 professional man-years in applied and developmental research on fermented foods from cereal grains, and 70 professional man-years in applied and developmental research on feed supplements.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Basic Research on Fermentation Processes

1. ARS Culture Collection maintenance and service. As of January 1, 1962, a total of 9,983 organisms were in the permanent collection of pure authentic cultures. This represents an increase during the past two years of 1,100 cultures. Some 3,187 culture transfers were distributed to domestic institutions and 1,425 to foreign ones.

After several years of negotiation, an exchange of nine streptomycete strains was made with Dr. G. F. Gause, Academy of Medical Sciences, Moscow, Russia. Also by exchange, 6 mold cultures were obtained from Poland and 53 strains of bacteria from Czechoslovakia, including a number of strains from insects. To prevent loss or destruction of the more important ARS Culture Collection strains, duplicate cultures are being stored at Olustee, Florida. A total of 1,749 strains is now there.

A total of 314 yeast cultures have been received from the National Institute of Agronomic Research, Madrid, Spain, and the University of Allahabad, India, has sent a number of unusual Mucoraceous fungi.

2. Bacteria investigations. Taxonomic studies on actinomycetes under a cooperative agreement with the Society of American Bacteriologists (now known as American Society for Microbiology) have now been completed. During the past 3 years some 2,000 strains were categorized into groups using a new scheme. About 1,000 of these strains were characterized by straight to flexuous fruiting bodies and were selected for more intensive study. Partial results were obtained on about two-thirds of these strains. Data collected on 129 strains were analyzed by ARS Biometrical Services to determine the merits of classification based on correlations among morphology of fruiting bodies, color of aerial mycelia, selected physiological tests, and nature of antibiotics produced. Certain patterns of correlation appear to exist, but more definitive information on antibiotic production is needed.

The first part of an investigation of a taxonomic group of streptomycetes, comprising about 50 antibiotic-producing strains, has been completed by determination of customary characteristics and antibiotic spectra.

Research on Pseudomonas is in abeyance because of assignment of the limited number of available qualified personnel to more urgent work. Some 27 strains of Pseudomonas, including a new species, have been received for future study. A group of 26 strains has been sent to Louisville University for use in a cooperative serological study of taxonomic relationships.

3. Yeast investigations. Following the discovery of sexual agglutination of certain genera of yeasts, described in the 1960 report, work was initiated that has as its objective a comprehensive study of this unusual phenomenon as a basis for developing new yeasts and new processes for fermentative conversion of cereal grains into new products. Blocking of sporulation is found in siblings of almost all strains of diploid species of Hansenula that possess sexual agglutination but blocking is absent in siblings that lack sexual agglutination. The function of blocking therefore was investigated. Siblings obtained from a strain isolated in Illinois were sexually agglutinative when mated among themselves, and the bisexuals they formed on mating were sterile. The same was true for siblings from a strain from Japan, but when mating types from the Illinois strain were mated with those of the Japanese strain, the resulting bisexuals were capable of producing sexual spores. Similar results were obtained with other strains from less widely separated geographic areas. Increasing degrees of inbreeding between the Illinois strain and one from Louisiana have shown increasing strength of blocking in the crosses. The discovery of spore blocking in Hansenula may enable the

production of stable, inbred strains incapable of sexual reproduction, that may have value for fermentative production of new chemicals or for use in assaying vitamins and antibiotics. A primitive form of sexual agglutination was found in two genera of related yeasts, Hansenula and Pichia. The process is a mechanical one in which the yeast cells produce tubes or filaments that connect cells directly together. Study has begun on a new family of yeasts believed to be predecessors of the important Basidomycete and Ascomycete classes of fungi. The new family differs from all other fungi in producing no sexual spores, although strong sexual reactions are observed.

4. Mold investigations. Studies were made of the sexual behavior of Absidia species. One species, A. cylindrospora, exhibited a hitherto unknown type of mating reaction. A "+" mating type reacted with some "-" types but not all. No explanation of this behavior is evident unless some of the more complex sexual situations associated with higher fungi are present. This work has also resulted in discovery of the sexual stage in two species of Absidia and in discovery of a new variety of A. blakesleeana. Taxonomic studies revealed a species, A. verticillata, that is parasitic on other Mucorales. Of 30 selected Mucorales, 10 species in 6 genera proved to be hosts of the parasite. So far as is known, this is the only parasitic Absidia.

Success was achieved in germinating spores of three strains of Rhopalomyces sp. at high pH's on a special medium. The first pure cultures in existence of this microorganism are, therefore, now available. It was shown that this fungus parasitizes and kills the eggs of certain nematodes but does not affect adults. Now that it can be grown in pure culture, its parasitic properties and potential value to agriculture can be studied.

Some 50 new cultures were added to the collection of fleshy fungi, making a total of about 450 since inception of the project. Yields of ramulosin, the seed germination inhibitor isolated from cultures of Pestalotia ramulosa, have been increased to nearly 16 percent based on glucose. Three species of Calvatia were found to yield an orange crystalline product. Preliminary characterization suggests a secondary or tertiary amine. Mouse tumor tests done at Battelle Memorial Institute in connection with the cancer testing program of the National Institutes of Health on 50 filtrates were negative, but 5 showed some promise in cytotoxicity tests at the University of Miami. Analysis of several promising filtrates showed that one contained mannitol and that three others contained polymeric materials as the chief non-glucose products. Analytical and cultural procedures for exploring fleshy fungi have been reexamined and modified for use in future work. Further study and reconsideration of results to date with 120 cultures showed that 26 percent had conversion characteristics warranting additional investigation.

5. Microbiological processes and products. Exploratory studies were made to discover new microorganisms that would give increased yields of hydroxyl-containing products by fermentation of a medium containing unsaturated long chain fatty acids. Optimum results were obtained with a Pseudomonas species isolated from oily residues found in a soybean oil processing plant. With this organism, oleic acid was converted to 10-hydroxystearic acid in yields of 14 percent with a medium containing 1 percent of oleic acid. The identity of the product was established by chemical analysis, determination of physical properties, and study of its behavior in a mass spectrometer. These results demonstrate production of a potentially useful industrial product from oleic acid by a highly specific microbial transformation. However, yields were too low to give the process practical significance, and it appeared unlikely that much higher yields could be realized. Furthermore, since the fermentation was aerobic, it could not be used satisfactorily with polyunsaturated fatty acids because of the ease with which they undergo autoxidation. Work on hydroxylation has been completed. Similar work has been initiated, however, on fermentative conversions of unsaturated fatty acids to amino derivatives. Selected yeast cells were incubated in a medium containing sodium fumarate and ammonium chloride. Chromatographic analysis of amination medium showed the presence of aspartic acid, thus demonstrating the ability of microorganisms to aminate the double bond of an organic acid. 4-Amino-hexanoic acid was synthesized, and its chromatographic R_f value and color reaction with ninhydrin were determined. Based on this information a screening procedure was devised whereby ability of organisms to aminate the model compound 3-hexenoic acid can be detected. Thirty organisms were screened with negative results. Further screening studies are in progress.

In the Pioneering Laboratory for Microbiological Chemistry studies on the enzymatic pathway by which pentoses are oxidized in Pseudomonas fragi revealed that while intermediates are the same for each pentose studied, the enzymes involved are different. The particulate aldose dehydrogenase, a constitutive enzyme, was unusual in that it oxidized glucose to glucono-γ-lactone whereas previous known enzymatic or chemical reactions yielded the δ-lactone. By use of immunological techniques, it was observed that in photosynthetic bacteria the photophosphorylation reaction could be interrupted by reagents capable of opening and blocking disulfide bonds. This observation resulted in discovery of an enzymatic, light-activated, disulfide reducing system in the bacterial particles. This system, first of its type to be found in photosynthetic particles, promises to be useful for studying relationships between particle structure and catalytic activity. Preliminary results of recently initiated studies of the mechanisms involved in yeast agglutination revealed that disulfide splitting reagents inactivated the agglutinating ability of one strain of yeast, whereas acid, base, and hydrogen bond-breaking reagents were more effective with a second strain. Chemical structures have been determined

for several metabolites. These include tetraacetylphytosphingosine (an extracellular lipid), ramulosin (an inhibitor for seed and fungus spore germination), and fonsecin (a yellow pigment).

At the University of Helsinki, Finland, nucleotides from radioactive yeast (Torulopsis utilis) were isolated and fractionated by chromatography on Dowex-1. The fractions were further separated by means of two-dimensional radio paper chromatograms and other techniques, enabling identification of the following compounds or mixtures: diphosphopyridine nucleotide; adenosine monophosphate; cytidine monophosphate; guanosine monophosphate + cytidine diphosphate; uridine monophosphate + inosine monophosphate + adenosine diphosphate; uridine diphosphate; cytidine triphosphate; adenosine triphosphate; guanosine triphosphate; uridine triphosphate; cytidine diphosphate - sugar; guanosine diphosphate - mannose; triphosphopyridine nucleotide.

With the possible exception of a substance tentatively identified as cytidylmonophosphoethanolamine, no new compounds were discovered. However, the following compounds identified have not been previously found in yeast: cytidyldiphosphocholine, cytidyldiphosphoglycerol, and cytidyldiphosphoethanoalamine. It is hoped ultimately to apply the knowledge gained in this research to the analysis of microbial systems producing polysaccharide gums, phospholipids and other products having potential for industrial use.

Research at the University of Milan, Italy, on fermentation of glucose to 5-ketogluconic acid has disclosed that the oxidative and decarboxylative functions of Acetobacter cells can be separated in cell-free extracts and independently characterized. Results of this work may disclose means of controlling their respective enzymic activities and, in turn, lead to more effective fermentations.

B. Industrial Chemicals

1. Microbial polysaccharides. Work has been completed on the team project on microbial polysaccharides.

Polymer B-1459. This polysaccharide is now being produced in the pilot plant by three industrial companies and marketed by one of them. To achieve maximum utilization of B-1459, its price should be as low as possible. Consideration has been given therefore to improvement of the process and to possibilities of use of partially purified fermentation liquors, thus avoiding isolation of purified solid polysaccharide. Studies on process improvement involved investigation of a new method for precipitation of polymer and development of a less costly fermentation medium. It was discovered that a commercially available quaternary ammonium salt would precipitate the polymer. A successful process based on this reaction was devised. It permits recovery and recycling of the quaternary salt and eliminates the

relatively expensive methanol precipitation procedure. It was also shown that products, which, although impure, should be useful in applications such as oil-well drilling muds and flooding agents for secondary petroleum recovery, can be obtained by drum and spray drying of fermentation broths. Revised cost estimates show that selling prices of various industrial grades of drum- and spray-dried polymers would range from 50 to 71 cents per pound. Viscosities of solutions of these impure products are equivalent, on the basis of actual polymer content, to those of solutions of methanol-precipitated polymer. For solutions of equal viscosity, those from the impure products would cost 60 to 70 percent less than those from methanol-precipitated polymer. Structural studies have revealed the presence of pyruvic acid in polysaccharide B-1459 as well as in several other Xanthomonas polysaccharides. It is possible that the presence of this unique constituent may contribute to the unusual viscosity stability of B-1459 to heat and inorganic salts.

Polymer B-1973. This product was successfully produced in the pilot plant by methanol precipitation in yields of 40 percent, and 5 pounds was prepared for industrial evaluation. It can also be recovered easily from the fermentation broth by precipitation with a quaternary ammonium salt, but the quaternary salt can not be completely removed from the complex by washing. Viscosity measurements indicated unusual response to salts. Addition of 1 percent of calcium or sodium chloride to 1-percent solutions of B-1973 doubled the original viscosity, but there was only a slight decrease in viscosity when salt concentration was increased to 25 percent. Addition of 0.05-0.50 percent of aluminum sulfate to a 1-percent polymer solution caused gelation, whereas higher concentrations decreased viscosity to less than that of the original. The polymer contains glucose, galactose, and mannuronic acid (as K salt) in equimolar ratios and 25 percent by weight of acyl (as acetyl), indicating that polymer units may be triacetates. Three potentially useful forms of the polymer exist: native, autoclaved native and deacetylated. Each has individual properties of interest; e.g., autoclaved native polymer forms unsupported films of good tensile strength and excellent flexibility even when unplasticized.

Polymer Y-1401. Composition has been established as mannose:xylose: glucuronic acid (as potassium salt):acetyl in the ratio of 4:1:1:1.5. α-Linkages are indicated. Yields were about 40 percent in 20-liter fermentors and 16.5 percent in initial large pilot-plant runs. Although the properties of this polysaccharide are interesting, they are not as unusual as those of B-1459. For this reason further developmental work will be kept in abeyance pending evaluation of available sample material by industry.

Polymers B-2171 and B-1828 did not reveal properties sufficiently different from those of other polysaccharides to justify detailed investigation and developmental research.

Phosphomannans. Primitive yeasts of the genus Hansenula and related genera elaborate a whole series of extracellular phosphomannans when grown on a medium containing glucose as a carbon source. For 30 strains from 10 species studied, phosphomannans with molar ratio of mannose:phosphate varied from 2.5:1 to 27.5:1; the more primitive yeasts produce the more highly phosphorylated gums while the more highly evolved yeasts in phylogenic lines yield gums with less phosphorylation. Mild acid hydrolysis of phosphomannans splits the crosslinking phosphate bonds yielding poly-mannosidic-6-phosphomonoesters. These relatively nonviscous polymers are acid-stable and act as dispersing agents, thus offering industrial potential.

Phosphomannan Y-2448 contains phosphorus:potassium:mannose in the molar ratio of 1:1:5. It is made up of chains of about 100,000 molecular weight and has a light-scattering molecular weight of about 16 million. The chains are crosslinked by hemiacetal phosphate bonds that are acid labile.

The structure of Y-1842 phosphomannan can now be described with considerable certainty as an oligosaccharide phosphoryl polydiester in which every fifth disaccharide unit contains an appended α-mannosyl residue. This structure can be explained by a simple, plausible scheme of biogenesis based on guanosinediphosphate mannose as the sole precursor. The mechanism would be applicable to all other phosphomannans.

Over 150 samples of phosphomannan Y-2448 have been distributed, and nearly 200 requests for information on phosphomonoesters have been received.

To encourage use of cereal grains as substrates for fermentative production of industrial chemical and other products, inexpensive media based on grain are needed. Preliminary cost studies indicated that enzymatically hydrolyzed corn should yield a medium containing fermentable glucose at a cost one-half that of commercial corn sugar. Such a medium, it is believed, might prove to be competitive with molasses. Research was, therefore, initiated to investigate this possibility. Initial experiments have revealed conditions permitting 95-percent conversion of starch to glucose.

2. Fermentative products from flour and gluten. Investigations on production of polyglutamic acid and on deamidation of wheat gluten have been completed. The polymeric product obtained by liquefication of wheat gluten with strains of Bacillus subtilis was found to be a mixture of polyglutamic acid and carbohydrate material that may have come from starch in the crude wheat gluten used. Methods were developed for isolation and purification of the polyglutamic acid. With fermentation times of 24 to 48 hours, the yield of polymer was equivalent to 33 to 42 percent of the glutamic acid in gluten. The polymer

contained 60-95 percent of D-glutamic acid, the remainder presumably being the L-isomer. In the pilot plant, polyglutamic acid was successfully produced in 20-liter fermentors in yields representing 15-17 percent conversion of glutamic to polyglutamic acid. Yields were low because of excessive foaming. For economic reasons, fermentative production of polyglutamic acid does not appear attractive at the present time. However, predicted excess industrial capacity for fermentative production of glutamic acid may require industry to seek new outlets for this acid. Hence industrial interest in conversion of glutamic acid to polyglutamic acid could develop.

Although research on deamidation did not result in products potentially useful to industry, good sources of proteolytic enzymes were developed. The proteolytic enzyme of B. subtilis NRRL B-2612 was isolated in highly purified condition. It was found that the viscosity of gluten could be effectively and controllably reduced by enzyme action. The products still had considerable molecular size and were quite uniform. These results suggest possibilities for enzymatic modification of wheat flour to obtain paper coatings, adhesives and related products. Research has been undertaken to explore these possibilities.

3. Fermentation acids. In the production of vital gluten from wheat by the batter process, a byproduct is a slurry (starch milk) containing starch, soluble sugars, and soluble nitrogen compounds. This starch milk may be used as a source of starch or fermented to beverage alcohol. It would be economically more advantageous, however, to convert starch milk to a valuable industrial product such as citric acid. Initial work showed that the starch must be hydrolyzed prior to sterilization in order to avoid gelatinization. A liquefication procedure was developed for this purpose based on use of a heat stable α-amylase enzyme. Treatment of starch milk with the enzyme for 30 minutes at 78° C. thinned out the slurry completely, and the product remained liquid during pressure sterilization. Yields of 90 percent of theory (based on starch consumed) were obtained when the manganese content was increased to 20 p.p.m., the value in the wheatberry.

The role of added methanol in fermentation of starch milk with a specific strain of Aspergillus niger has been investigated. During the growth period of the mycelium, methanol is metabolized. After the mycelium is fully developed, no methanol is utilized, indicating that it does not enter into the formation of citric acid. If no methanol is added to the medium, this strain gives only a 10-percent weight yield. The effects of added manganese and methanol make fermentative conversion of starch milk to citric acid economically and technically feasible. Present estimates indicate that carbohydrates in the starch

milk of the batter process can be made available at about the same cost as sugars in high-test molasses. The strain ferments the starch milk without the formation of oxalic or gluconic acid. Currently citric acid sells for 28 cents per pound and oxalic acid is 18 cents per pound.

Work on development of a process for recovery of α-ketoglutaric acid from fermentation liquors, which was effected at Augustana Research Foundation, Rock Island, Illinois, has been completed. A successful process was devised that should be readily adaptable for industrial use. Approximately 80 percent of α-ketoglutaric acid present in the fermentation liquor can be recovered as calcium salt approaching 100-percent purity. Essential steps in the process are extraction of α-ketoglutaric acid with cyclohexanone and precipitation of the calcium salt. Even higher yields would be expected in an actual industrial application of the process where continuous extraction and multiple crystallization would undoubtedly be utilized.

C. Biological Pesticides

1. Biological insecticides for Japanese beetle. A significant advance was made in research, conducted with the cooperation of the Entomology Research Division and the Plant Pest Control Division, on development of large-scale production of "milky disease" spores for controlling Japanese beetle. The viability of cells of Bacillus popilliae has been improved substantially so that cells produced in liquid media remain alive for a period of time that may be sufficient to allow sporulation under favorable conditions. The principal change in procedure that permitted improvement in viability was increase of buffer (K_2HPO_4) to 0.6 percent.

A semisynthetic liquid culture medium was developed for the propagation of B. popilliae in which a number of cells show early stages of spore development. Sporelike forms have been obtained repeatedly when B. popilliae is grown on the surface of a solid medium containing sodium acetate but no glucose. No spore forms were obtained if lactate or glucose were present.

Studies on grub blood and live grubs are vital to this work because grub blood is, so far, the only liquid medium in which sporulation of milky disease organisms occurs. The amino acid composition of hemolymph from diseased Japanese beetle larvae was found to contain more lysine, glutamic acid and ornithine and less arginine, proline, alanine, serine and tyrosine than does hemolymph from healthy larvae. Glutamine is apparently hydrolyzed in deceased larvae. Hemolymph of normal grubs was about 33 percent saturated with oxygen in respect to air, whereas in infected grubs this value ranged from 5 percent (spores present) to 15 percent (only vegetative cells present). Through the

cooperation of General Dynamics Corporation, quantitative elemental analysis of normal grub hemolymph has been obtained.

At Michigan State University, information on the metabolic pathway for glucose in B. popilliae is being developed. One preliminary result of interest is that low oxygen supply may result in production of three times more lactate than acetate from glucose, while with high oxygen supply no lactate is produced.

2. Insect attractants. In cooperation with Entomology Control Division field stations, a total of 520 cultures, representing 149 species of yeasts, 63 species of bacteria, 126 species of molds, 47 species of actinomycetes and 135 cultures from 5 groups of fleshy fungi, were grown on grain-based media and tested as insect attractants either as whole cultures or as centrifuged supernatants. The fermenting whole culture product of the yeast Candida utilis, Y-900, appears to be the most promising. Lures prepared from this product were four times as effective as the standard lures for attracting Mexican fruit flies.

3. Plant antibiotics. Screening of 1,000 actinomycete isolates has been completed. About one-fourth of them produced antibiotics of interest and were further studied to increase yields. During the last two years of these studies, Crops Research Division conducted greenhouse tests on 184 fractions and filtrates for effectiveness against rust, anthracnose, downy mildew, bacterial blight, and mosaic of beans. Forty-three of these samples gave positive results against one or more of the five diseases. Comparative chromatography eliminated a number of isolates that produced known antibiotics. Appraisal of the overall results has reduced the number of choice strains to 20 for more intensive study.

The F-17 antibiotic mixture elaborated by Streptomyces cinnamomeus forma azacoluta Pridham et al., NRRL B-1699, has been resolved into the following:

F-17-A: Possibly a mixture of two or three antibiotics of unknown structure having antibacterial activity.

F-17-B: Duramycin, a polypeptide active against gram-positive rods and some yeasts and molds but ineffective against bean rust.

F-17-C: An antifungal heptaene complex having virtually no activity against bacteria, streptomycetes, or bean rust.

Antibean-rust factor: Highly active fractions were isolated, but the active agent was not characterized as a pure compound. Recent evidence suggests that the antirust factor(s) and the antibacterial factor(s) of F-17-A may be the same.

This work, which completes studies on the F-17 mixture, resulted indirectly in the discovery that phleomycin, an antibiotic discovered in Japan, was active against bean rust at a level of 0.17 p.p.m.

Work was begun on a screening program designed to find antibiotics effective against a selected group of plant fungi, specifically Alternaria solani, Fusarium oxysporum f. lycopersici and Mucor ramannianus. Since these test organisms can be cultured in the laboratory, more rapid and effective screening is possible than when greenhouse tests provide the only method of in vivo testing. Only materials showing the greatest activity against the test organisms will be submitted to Crops Research Division for further testing. Since inception of this work 358 streptomycete strains have been screened, of which 48 showed potential nonpolyenic antifungal activity. Further screening for stability reduced this number to 15 promising strains.

D. Feed and Food Products

1. Microbial carotenoids. Studies are being completed on the production of β-carotene by fermentation of cereal-based media. By use of new, more efficient strains of the organism Blakeslea trispora and addition of both a nonionic detergent and a specially purified kerosene fraction to the medium, yields of β-carotene were increased to 90 to 100 mg. per 100 ml. of medium. Other ingredients of the medium are hydrolyzed corn and soybean meal, animal fat, β-ionone, and thiamine hydrochloride. Replacement of hydrolyzed corn and soybean meal by whole corn and inexpensive, commercially available cottonseed embryos gave yields of 135 mg. per 100 ml. of medium. Also, replacement of β-ionone with citrus pulp gave yields of 102-109 mg. per 100 ml. Even though only 0.1 percent of β-ionone was used in the medium, this chemical is so expensive that its replacement by citrus pulp results in reduction of the estimated "cost to make" of the product from $42 to $32 per kilogram. Whereas previous yields indicated a process of marginal economics, current yields should provide the basis for an attractive industrial process. Six industrial organizations that are evaluating the process reported that they were able to equal or exceed the stated yields of product.

Further studies have shown that a variety of citrus products, including dry citrus meal and citrus essential oils, can replace β-ionone. The active principle of dried citrus peel that permits its substitution for β-ionone in fermentative carotene production can be eluted in a water-soluble fraction. The active precursor in grapefruit and tangerine oils was identified as limonene. The compounds 4-keto-β-ionone, 4-hydroxy-β-ionone and β,β-dimethylacrylic acid have been synthesized for study as precursors in xanthophyll production.

2. Vitamin B_{13}. A method for extracting large batches of distillers' dried solubles (DDS) for vitamin B_{13} activity yielded a preparation having animal growth activity. Mevalonic acid and orotic acid, both present in DDS, seemingly have been eliminated as contributing to B_{13} activity. Studies on the characterization of the vitamin showed that a fraction of "B^{+}_{13} concentrate," purified by high vacuum distillation, as well as the original extract, yielded upon acid hydrolysis a product containing about 14 amino acids. This research is conducted by the "Giuliana Ronzoni" Scientific Institute of Chemistry and Biochemistry, Milan, Italy.

PUBLICATIONS AND PATENTS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

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AREA NO. 6: FLAXSEED
INDUSTRIAL UTILIZATION OF LINSEED OIL

Problem. Traditional markets for linseed oil, the major drying oil produced and used in the United States, are threatened by growing use of synthetic products derived from nonagricultural sources. Thus, over the years 1950-1960, use of linseed oil in linoleum and oilcloth decreased from 107 to 22 million pounds because of displacement by synthetic materials capable of better performance. During the same period, consumption of synthetic products in protective coatings increased by 50 percent. Solvent-thinned, exterior house paints, the largest remaining outlet for linseed oil, are being displaced to a significant and growing extent by recently developed water-thinned, synthetic resin emulsion paints that have many advantages such as ease of cleanup, fast drying, and freedom from objectionable odors. Consumption of these synthetic emulsion coatings is estimated at 10 to 20 percent of the 100 million gallons of exterior house paints produced in 1960.

To restore the competitive position of linseed oil, new or expanded markets are urgently needed. Chemical modification of linseed oil offers opportunities to obtain new products that can compete with nonagricultural synthetic products and thus stop, or even reverse, the present downward trend in utilization of linseed oil. For example, development of satisfactory emulsions and water-soluble vehicles that combine the attractive properties of synthetics with the inherent protective advantages of oil-based finishes could enable linseed oil to recover lost markets in protective coatings, which were produced to the extent of nearly 670 million gallons in 1960. Chemical modification also should open entirely new outlets for linseed oil such as the organic chemical industry which now produces 15 billion pounds of end products and is expected to reach 25 billion pounds by 1965. To furnish a sound basis for chemical modification, an adequate program of basic research on linseed oil is required to furnish new leads and new concepts that will point the way to those products having the best chance for acceptance in the market place.

USDA PROGRAM

The Department conducts a continuing long-range program involving analytical, organic and physical chemists and chemical engineers engaged in basic research on the chemical reactions of linseed oil and its component fatty acids and in the application of the knowledge gained to the development of new or improved products for the chemical and protective coating industries.

The Federal scientific effort concerned with research on industrial uses for linseed oil totals 17.8 professional man-years. Of this number 7.4 is devoted to industrial chemical products and 10.4 to protective coating products.

The current program at Peoria, Illinois, does not include research specifically devoted to chemical composition and physical properties. During the reporting period contract research on phospholipids of flax and on the substances formed during after-yellowing of linseed oil films was discontinued.

Research at Peoria, Illinois, on industrial chemical products (6.5 professional man-years) involves exploratory studies to find new reactions and chemical derivatives and basic and applied research on cyclic fatty acids. During the reporting period research other than engineering studies on conversion of linolenic acid to cyclic acids containing 18 carbon atoms was discontinued to permit more intensive investigation of a newly discovered type of cyclic acid containing 20 carbon atoms. Research contracts on industrial chemical products (.9 professional man-year) are in effect at the University of Arizona, Tucson, Arizona, for studies on polymerization of vinyl monomers derived from cyclic and other fatty acids (.3 professional man-year) and at Battelle Memorial Institute, Columbus, Ohio, for evaluation of aldehyde products derived from linseed oil (.6 professional man-year).

Studies on protective coating products in progress at Peoria, Illinois, (9.0 professional man-years) include investigations on new polymers from linseed oil for use as water-soluble vehicles for coatings and basic and applied research on problems related to development of linseed emulsion paints. During the reporting period research, except for provision of samples to interested industrial companies, was discontinued on linseed vinyl ethers and their use as protective coatings. Research contracts on protective coating products (1.4 professional man-years) are in effect with the University of Southern California, Los Angeles, California, for basic physical chemical studies on linseed oil emulsions and pigment suspensions (.7 professional man-year) and with Kansas State University, Manhattan, Kansas, for research on the use of linseed oil to protect concrete (.7 professional man-year). During the reporting period contract research on evaluation of linseed vinyl ether coatings and on special linseed oil copolymers for emulsion paints was completed.

The Department also sponsors research (10.1 professional man-years) conducted by foreign institutions under grants of PL 480 funds. Research on chemical composition and physical properties (3.0 professional man-years) involves a grant to the Experiment Station for Fats and Oils, Milan, Italy, for studies on minor constituents of linseed oil. Research on industrial chemical products (3.0 professional man-years) is also conducted by this institution under a grant for the

investigation of products obtained by thermal polymerization of linseed and other polyunsaturated vegetable oils. Research on protective coating products involves a grant to the Paint Research Station, Teddington, England, for fundamental research on organometallic compounds as components of protective coatings (4.1 professional man-years).

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported no work on industrial utilization of linseed oil.

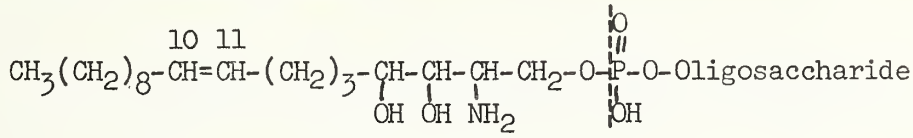
Industry and other organizations conduct interesting and significant research on linseed oil. Chemical research to develop new or improved products from linseed oil is conducted almost exclusively by the largest processors of flaxseed and by several of the largest manufacturers of protective coatings. A major share of the research of most of these companies concerns development of new protective coating products from linseed oil. In some organizations, research effort is devoted to development of other types of industrial products and to improvements in processing to achieve economies in recovering linseed oil from flaxseed or to obtain higher grades of oil. Very little basic research is done. Manufacturers of protective coatings, as well as many other industrial companies, endeavor to improve products based on existing materials available from linseed oil in order to meet competitive situations or to solve other problems incidental to company operations. Except for certain of the largest protective coatings companies, this activity, although large, does not appear to involve research to derive or develop new or improved products from linseed oil itself. Member companies of the National Flaxseed Processors Association support a fellowship at the Northern Division, Peoria, Illinois, for research on emulsion paints. Frequently, the companies participate in formal or informal cooperation with the Division for testing and evaluation of experimental products developed at the Division. With the exception of the cooperative work, the nature of the processes and products studied and the results of the research are kept confidential by the companies. Estimated annual expenditures are equivalent to approximately 2.0 professional man-years in basic research, 5.0 professional man-years in development of industrial products other than protective coatings, 5.0 professional man-years in improvement of processing technology, and 35 professional man-years in development of protective coatings products.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Flaxseed phospholipides. Final studies at the University of Illinois on characterization of flaxseed phospholipides have revealed

additional details of the composition of the oligosaccharide components of flax phytoglycolipides:



Phytosphingosine moiety

Unlike corn, soybean, and peanut phytoglycolipides, flax phytoglycolipide is a mixture. Upon hydrolysis two oligosaccharides were obtained:

(a) -Inositol - glucuronido - glucosamine { galactose, arabinose } mannose

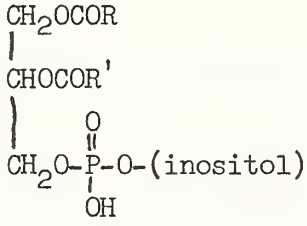
This oligosaccharide moiety was also found in soybean phytoglycolipide.

(b) Oligosaccharide with molecular weight greater than 2,000, yielding on hydrolysis inositol, galactose, arabinose, and fucose. Fucose was not found in soybeans or corn and may be unique for flax.

Thus it appears that a variety of phytoglycolipides may exist in seed phosphatides, consisting of a series of related molecules with different oligosaccharides.

In flax, the phytosphingosine moiety has a <u>trans</u> double bond at position 10,11. The soybean product has this double bond at the 11,12 position, while in corn the phytosphingosine base is saturated.

Linseed phosphatidyl inositide has the following structure:



R and R' are fatty acid moieties. Major components are palmitic and oleic acids, with minor amounts of stearic, heptadecanoic and higher unsaturated fatty acids. This inositide was readily prepared in pound lots. Samples were distributed to interested outside investigators for study of possible applications in medicine, enzymology and microbiology. At the Northern Division, the substance was found to

improve flavor and oxidative stability of edible soybean oil in concentrations as low as 0.01 percent.

2. After-yellowing of linseed oil films. It was reported in 1960 that the sequence of reactions leading to formation of yellow substances in linseed oil films could be blocked by aldehydes containing α-methylene groups. To prevent or decrease after-yellowing of films under practical conditions, aldehydes were needed that would have the required structure and that would be nonvolatile and adequately soluble in the oil. Two types of aldehydes were found that met these criteria:

1. Glycol aldehyde esters, $\begin{matrix} CHO \\ | \\ CH_2OCOR \end{matrix}$

2. Monoglyceryl esters of hemialdehydic acids, $\begin{matrix} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OCO(CH_2)_nCHO \end{matrix}$

The following table shows some of the results obtained with azelaaldehydic acid monoglyceride (AM) (type 1) and glycolaldehyde palmitate (GAP) (type 2).

	Onset of yellowing[a] (hrs.) with stated inhibitor			
Oil	None	0.5% AM	0.5% GAP	10% GAP
Menhaden	33	112	--	--
Linseed methyl esters	70	122	70	168
Safflower methyl esters	140	159	--	--

a/ Accelerated tests.

Compounds of type 1 appear to be more effective than those of type 2. This research, which was conducted by the Hormel Institute, University of Minnesota, Austin, Minnesota, has been completed.

3. Minor constituents of linseed oil. Analysis of unsaponifiables of linseed oil has revealed the presence of at least 43 paraffin hydrocarbons (normal and branched), terpene and other alcohols, waxes, sterols, esters, and squalenes. This work is being done at the Experiment Station for Fats and Oils, Milan, Italy.

B. Industrial Chemical Products

1. Cyclic acids. Cyclization: Conditions and catalysts for alkaline cyclization of linolenic acid of linseed oil were studied intensively.

Significant improvements in this cyclization process, which yields cyclic acids containing 18 carbon atoms (C-18), were achieved. t-Butoxide catalyst in t-butyl alcohol converted 83 percent of the linolenic acid present in linseed fatty acids to C-18 cyclic materials. When cyclization was conducted in ethylene glycol with the sodium salt of ethylene glycol as the catalyst, yields were equivalent to the best previously found. With sodium hydroxide as catalyst and ethylene glycol as solvent, a yield of 46 grams of cyclic acid per 100 grams of linseed oil was obtained when the reaction was conducted at 295° C. under ethylene. This yield is 92 percent of theoretical based on linolenic acid. Pilot-plant runs indicate up to 98-percent yields based on linolenic acid with ethylene present. Cyclization has also been successfully performed as a continuous process in a specially designed reactor. Yields of cyclic acid were somewhat lower than those from batch operation, but formation of undesired polymers was substantially reduced. Continuous processing with ethylene present gave yields about equal to those from batch runs without ethylene. The increased yield of cyclic acid in the presence of ethylene was found to result from addition of ethylene to the conjugated fatty acids formed under the alkaline conditions of the reaction. By reaction at 260° of ethylene with a preformed conjugated acid, 9,11-trans,trans-octadecadienoic acid, an adduct was obtained in 92-percent yield (no catalyst required). This adduct is a new cyclic acid product containing 20 carbon atoms (C-20). At 3,000 p.s.i. alkaline cyclization of soybean oil and safflower oils in the presence of ethylene gave yields of C-20 cyclic acids amounting to 40 g./100 g. oil and 57 g./100 g. oil, respectively.

Derivatives: Accelerated yellowing tests showed that oil-modified alkyd resins modified with cyclic acids yellowed less than alkyds modified with soybean or safflower oils. Other evaluation tests showed that modification of alkyd resins with cyclic acids improved drying times, hardness, and resistance to water and alkali over resins modified with natural vegetable fatty acids and oils.

The amides prepared from cyclized linseed fatty acids (unpurified except for removal of polymers) (I) and from hydrogenated cyclic acids (II) have low melting points (33° and 43°, respectively) and high solubilities in carbon tetrachloride and benzene compared to hydrogenated tallow amides. The amide of II exhibited better compatibility with synthetic resins than did oleamide. The nitriles of I and II are liquids having low freezing points (-21° and -25°, respectively). The nitriles of II were compatible with vinyl chloride-acetate copolymer (PVCA). Sheets of PVCA plasticized with nitrile were soft and flexible, but more extensive evaluation in this application appears desirable. The ethenoxylated amide and quaternary amine derived from I have surface-active properties comparable to similar fat-derived substances. The diethanolamides of I and II have very low oil-water interfacial tensions and were very nearly equivalent to

coconut diethanolamides in surface-active properties. Laboratory studies on production of C-18 cyclic acids by the alkali-catalyzed reaction in the absence of ethylene have been completed. Engineering and pilot-plant investigations are continuing. Information and samples of C-18 cyclic acids have been made available to interested industrial companies.

2. Polymerization of vinyl compounds. Studies were initiated at the University of Arizona on the use of vinyl esters of hydrogenated cyclic acid, vinyl 9,10-dichlorostearate, vinyl 9,10,12,13-tetrachlorostearate and other fatty vinyl esters as internal plasticizers for polyvinyl chloride. Polyvinyl chloro derivatives have been obtained that have lower melting and brittle temperatures than had previously been obtained with fatty products.

3. Glyceride polymers. Characterization of glycerides obtained at various stages of thermal polymerization of soybean and linseed oils is being investigated at the Experiment Station for the Fats and Oils Industry, Milan, Italy. During the past year a bibliography of 227 pertinent references was assembled. Forty polymerizations of linseed oil were conducted at various temperatures and for various lengths of time. A portion of each sample was chromatographed on columns charged with ashless cellulose powder. Materials elutable with methanol were converted to methyl esters and examined by gas chromatography. Also, ultraviolet and infrared spectra were obtained on the eluted oils. Since the chromatographic procedure separates materials that appear to be unpolymerized, it is noteworthy that this methanol-elutable material was, in every instance, formed in about 20-percent yields. Definite conclusions have not yet been reached regarding the significance of this observation or of the other data so far collected.

C. Protective Coating Products

1. Vinyl ether coatings. Contract research at Battelle Memorial Institute covering end-use evaluation of selected conjugated linseed vinyl ether polymers showed that although very significant improvement in film properties had been achieved during the course of the work, even the best polymers had one or more deficiencies. Improved curing properties, better resistance to forming operations (as in making can ends from coated sheet metal), and increased compatibility with other commercially available resins would be desirable.

A short time before conclusion of the contract work, methods for styrenating vinyl ether polymers were discovered. Only a few tests on these could be run at Battelle, but the results were encouraging because they showed that the styrenated polymers had improved properties, including compatibility with certain commercial resins. However, these styrenated polymers were stable only for short periods, eventually gelling. A technique was developed that permits essentially

complete incorporation of up to 70 percent of styrene into low-molecular-weight (1,500) conjugated linseed vinyl ether polymers. Comparable results were obtained with various conjugated linseed vinyl ether copolymers, including those made with cyclic comonomers. Basis of the technique is use of dipentene either as the only or as the predominant reactive solvent for styrenation. It was further demonstrated that a vinyl ether polymer could be prepared and styrenated without isolation of the intermediate polymer. Further evaluation of both styrenated and unstyrenated vinyl ether copolymers gave results indicating that research on fatty vinyl ether polymers has very nearly achieved a material that would be definitely interesting to industry. A major problem is to attain adequate resistance to the forming operation. Experience with the recently tested samples suggests that some combination of the following monomers might succeed: conjugated linseed and soybean vinyl ethers, isobutyl vinyl ether, cyclopentadiene, and styrene. Research on vinyl ether coatings has been discontinued except for preparation of samples for detailed evaluation by two cooperating commercial organizations.

2. Emulsion paints. Research on linseed emulsion paints for exterior use on wood was undertaken in 1959 in cooperation with the National Flaxseed Processors Association as a means of retaining the market for linseed oil in competition with synthetic emulsion house paints. The results of this program have been eminently successful. During the past year, two companies began marketing emulsions from which stable paints containing zinc oxide (for mildew resistance) can be formulated. Another company is planning to market a linseed oil emulsion. Current reports are that some 50 paint manufacturers are marketing exterior house paints prepared with these emulsions as vehicles. Companies marketing these emulsions have publicly acknowledged the importance of the Department's research to the success of this development. Several of the more significant results of the Department's research were described in the report for 1960. These included development of paints that dried to touch in 15-30 minutes and could then be recoated; use of chelating agents to minimize destabilization of paints by metallic driers; use of hydroxyethyl cellulose to improve surface-dry and water resistance; and development of new, reactive emulsifiers derived from linseed oil.

During the present reporting period, further important contributions were made including development of stable paints containing reactive zinc oxide pigment; information on variables affecting brushing, leveling, wet-edge and other application properties; demonstration of previously unreported interactions between titanium oxide and zinc oxide in aqueous slurries that lead to unstable viscosity and pH; and discovery of several means for preventing such interactions, e.g., proper addition of ethylene glycol, correct concentration of organic dispersing agent or use of phosphate dispersing agents (tests uncompleted). Despite the success so far achieved, further improvements in linseed

emulsion paints are needed to meet present and future competition from synthetics. Problems relating to application characteristics, storage stability, and performance over bare wood, especially redwood and red cedar, remain to be solved.

A reproducible ultracentrifugal method was developed at the University of Southern California for quantitative measurement of stability of oil emulsions. The method has been further refined and its use in evaluating an important variable affecting emulsion stability has been demonstrated. Thus it was shown that stability of a Nujol-water emulsion stabilized with sodium dodecyl sulfate (SDS) increases with SDS concentration until the critical micelle concentration is reached after which stability was independent of concentration. Results obtained from experiments on centrifugation of emulsions suggest that interdroplet water may be squeezed out from between the oil droplets during centrifugation without coalescence necessarily ensuing and that this water may then gradually diffuse back on standing. Studies on the interaction of zinc oxide and surfactants showed that when alkali was added to a solution containing zinc chloride and SDS, a precipitate was obtained at a pH lower than that at which zinc hydroxide precipitates under similar conditions but in the absence of SDS. X-ray diffraction patterns revealed that the precipitate was not zinc hydroxide but might be a basic zinc dodecyl sulfate of variable composition. When Tamol 731 (a dispersing agent) was substituted for SDS, no precipitation occurred even at pH 10, indicating that zinc ions are bound by Tamol. A number of different Nujol emulsions containing zinc oxide were prepared. X-ray diffraction showed that the suspended species is zinc oxide and not zinc hydroxide. With acid sols, where the zinc oxide carries a positive charge, no emulsions could be stabilized and pigment became oil-wet. With an alkaline sol (pH 12) stability of dilute oil-water emulsions increased as the amount of zinc oxide was increased. Demonstration of interactions between zinc oxide and surfactants has important implications in relation to stability of emulsion paints containing zinc oxide. Thus, such interactions might result in reduction of amounts of emulsifiers and dispersing agents needed for stability to ineffective levels or in conversion of zinc oxide into some other chemical entity having different requirements for dispersion and stabilization.

At Stanford Research Institute, acrylate groups have been successfully introduced into bodied linseed oil (M-37 oil). This result was accomplished by two methods: (1) methanolysis followed by reaction with acrylyl chloride, or (2) glycerolysis with sodium glyceroxide followed by reaction with acrylyl chloride. A method for grafting methyl acrylate onto M-37 linseed oil also was developed; the product is soluble but rapidly crosslinks in air to an insoluble product. The graft copolymer was made into an emulsion paint but the oil gelled too readily for best results and films remained tacky for extended periods. This research has been completed.

3. Organometallic compounds in paints. At the Paint Research Station, Teddington, England, products having good drying properties (set-to-touch in one-half hour; no drier) have been obtained by reacting oleyl-linoleyl (or linoleyl-linolenyl) acetoacetate with aluminum isopropoxide and then linseed fatty acids. Other types of compounds under investigation include reaction products of titanium isopropoxide, triisopropyl borate and aluminum isopropoxide, with ethanolamides of oleic and linoleic acids, with fatty anilides, and with fatty-substituted amino alcohols.

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AREA NO. 7: SOYBEANS
FOOD AND INDUSTRIAL USES FOR SOYBEAN OIL

Problem. Soybean oil is now the major edible oil of the United States and the most important source of nutritionally important linoleic acid. However, this oil contains an unstable component (linolenic acid) that limits its use as a liquid oil both domestically and in foreign markets. It is estimated that in 1961 over 3.2 billion pounds of soybean oil (about 90 percent of total domestic use) was consumed in edible products, of which somewhat more than two-thirds was consumed in hydrogenated form as margarine and shortening. However, production of soybeans continues to increase rapidly and is expected to be about 700 million bushels in 1962.

The most promising outlets for oil from this ever-growing production of soybeans appear to be in foreign markets as edible oils and fats and in domestic industrial uses. The potential market for vegetable oils imported by Europe is estimated at 7.5 billion pounds by 1975. For soybean oil to capture a growing share of this market, more information is needed to show how to eliminate unstable linolenic acid without loss of nutritive value, to determine the extent to which minor constituents influence flavor and other properties of the oil, and to discover methods for modifying hydrogenated soybean oil to achieve desired functional properties such as melting point and texture. This information would also serve as the basis for improving soybean oil for domestic use both as a liquid oil and in its hydrogenated forms. Some additional consumption in the United States might be anticipated because of extended utility resulting from these improvements, even though consumption of edible fats and oils mainly increases with population growth. To achieve the objective, a broad program of basic and applied research is required to provide more knowledge of the properties of linolenic acid and of minor constituents of soybean oil; of the changes that take place in these and other components during oxidation, hydrogenation, and heating; of the effects of these changes on flavor, nutritive value, stability, and other qualities of the oil; and of the effects of modification of glyceride structure on functional properties of hydrogenated forms of soybean oil.

As an industrial oil, soybean, like linseed oil, is faced with growing competition from synthetic products derived from nonagricultural sources. As an industrial source of linoleic acid, soybean fatty acids must also compete with tall oil fatty acids, a byproduct of paper manufacture. The best opportunity for increasing industrial applications of soybean oil appears, therefore, to be development of products that retain the glyceride structure of the oil. Thus, aldehyde oils, a recent discovery of Department scientists, appear to have a promising future, if current research and development is successful, in the

3-billion-pound market for resins, fibers, coatings, plastics, plasticizers, pesticides, and paper and textile chemicals. To achieve the potential industrial value of aldehyde oils and other soybean glyceride products, more fundamental information is needed on reactions of soybean oil that will preserve the glyceride structure and on the physical and chemical properties of the products. Upon this basis, development of a wide variety of new, industrially useful products should be possible.

USDA PROGRAM

The Department has a continuing long-range program involving analytical, organic and physical chemists and chemical engineers engaged in basic and applied research on edible and industrial uses of soybean oil. A food technologist is also required by the program in connection with organoleptic evaluation of edible oils. Objectives of research on edible soybean oil are to identify undesirable flavor components of the oil, to develop basic information on the chemical changes and mechanisms involved in formation or suppression of these components and to apply the knowledge gained to the development of edible soybean oil having improved oxidative, thermal and organoleptic stability. Objectives of research on industrial utilization are to obtain new information on reactions of soybean oil and its components and to use this information to develop new or improved products for use by the chemical and other industries.

The Federal scientific effort for research on soybean oil totals 23.3 professional man-years. Of this number 6.0 are devoted to chemical composition and physical properties, 10.2 to edible utilization, and 7.1 to industrial utilization.

Research at Peoria, Illinois, on chemical composition and physical properties (6.0 professional man-years) is concerned with isolation and identification of components affecting flavor stability of soybean oil and with basic studies on autoxidation of this oil.

Research at Peoria, Illinois, on edible utilization of soybean oil (9.0 professional man-years) involves basic and applied studies on selective hydrogenation as a means of stabilizing soybean oil by removal of linolenate. During the reporting period research on use of phosphonic acids to stabilize soybean oil was discontinued. A research contract (1.2 professional man-years) is in effect with Armour Research Foundation, Chicago, Illinois, for preparation and evaluation of catalysts for selective hydrogenation.

Research at Peoria, Illinois, on industrial utilization of soybean oil (6.5 professional man-years) involves exploratory studies to find new reactions and products and basic and applied investigations of aldehyde oils and other aldehydic products. During the reporting

period research on Diels-Alder adducts from conjugated soybean fatty acids was discontinued. Exploratory studies on oxidative cleavage of unsaturated fatty acids of soybean oil were completed, and development work is being undertaken. A research contract (.6 professional man-year) is in effect with Battelle Memorial Institute, Columbus, Ohio, for preliminary evaluation of aldehyde oils and other aldehydic products from soybean oil in selected applications.

The Department also sponsors research on soybean oil (18.0 professional man-years) conducted by foreign institutions under grants of PL 480 funds. Research on chemical composition and physical properties (4.7 professional man-years) involves grants to the Institute for Fats and Their Derivatives, Seville, Spain, for research on removal of trace metals from soybean oil with ion-exchange resins (2.0 professional man-years) and to Gdansk Polytechnic, Gdansk, Poland, for studies on soybean sterols and their effect on stability of the oil (2.7 professional man-years). Research on edible utilization (6.0 professional man-years) is conducted under grants to the University of Granada, Spain, for studies on the effect of processing on frying quality of soybean oil (3.8 professional man-years) and to Tokyo University, Japan, for research on hydrogenation of soybean oil (2.2 professional man-years). Research on industrial utilization (7.3 professional man-years) involves grants to the University of Helsinki, Finland, for studies on separation of pure fatty acids from mixtures such as soybean fatty acids (2.3 professional man-years); Queen Mary College, University of London, England, for basic studies on alkaline cleavage of polyunsaturated fatty acids (2.0 professional man-years); and the Experiment Station for the Fats and Oils Industry, Milan, Italy, for research on oxidation with atmospheric oxygen to obtain new soybean oil derivatives (3.0 professional man-years).

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of .6 professional man-year, divided between subheadings as follows: chemical composition and physical properties, .5; industrial utilization, .1. A limited amount of effort is devoted to problems of the chemical composition and physical properties of soybean oil. Thermal polymerization, heat damage, and flavor reversion are special problems under study. Industrial utilization of soybean oil is the subject of an exploratory study at one location. This work involves testing new use ideas and determining their feasibility.

Industry and other organizations conduct a substantial research program on soybean oil. The predominant interest appears to be improvement of soybean oil for use in various edible products such as shortening, margarine, and salad and cooking oils. A considerably smaller, but still important, emphasis is placed on industrial utilization of soybean oil. Basic research on edible and industrial utilization of soybean oil

receives a moderate share of the effort. The research on industrial utilization is predominantly concerned with finding new industrial outlets rather than improvement of end-products containing soybean oil. In the edible field, however, research is directed both towards modification of end-products to meet competitive situations and towards basic improvements in processing the oil to make it more suitable for use in edible products. The companies have cooperated extensively in evaluation of edible and industrial products from soybean oil, and of processes for making them, developed by the Department. During 1961-62 the National Soybean Processors' Association is supporting basic research (about .3 professional man-year) at the Northern Division on flavor components of reverted soybean oil. This work was supported in 1960-61 by the Soybean Council of America. Except for cooperative research, the exact nature of the products and processes investigated by industry and the results of the research are kept confidential or patented by the companies. Estimated annual expenditures for research on soybean oil are equivalent to approximately 25.0 professional man-years in basic research, 110.0 professional man-years in applied, developmental, and product improvement research on edible products from soybean oil, and 40.0 professional man-years in applied and developmental research on new industrial products.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Flavor components. Studies showed that unsaponifiables by all methods of preparation were detrimental to cottonseed oil when added at levels equal to those present in soybean oil. (Cottonseed oil was used for these tests to avoid complications from inherent instability of soybean oil.) Indeed, some samples were detrimental to cottonseed oil at levels of one-half to one-third the amount present in soybean oil, and flavor scores typical of soybean oil were obtained.

Chromatography using methanol and methanol-benzene separated the unsaponifiables into three fractions of widely different polarity. Gas chromatography showed the fractions to contain 11, 4, and 5 components, respectively. Fraction I comprised 6 to 50 percent of the unsaponifiables and contained hydrocarbons and tocopherols; Fraction II comprised 18 to 60 percent, was heat sensitive, and was modified by ester saponification; and Fraction III comprised 20 to 40 percent and contained steroids. Autoxidation of the oil increased the nonpolar, hydrocarbon components of the unsaponifiables. Nonpolar components (I) were more detrimental to oxidative stability than more polar components (III). Purified stigmasterol and sitosterol were not detrimental to flavor or oxidative stability of cottonseed oil.

The presence of the following classes of organic compounds in flavor and odor components removed from refined soybean oil (obtained from beans by extraction) by high-vacuum stripping was observed: saturated and unsaturated hydrocarbons (methane, ethylene and higher homologs), alcohols, methyl and ethyl esters (up to C_8 in volatile fractions), aldehydes, acetals, methyl ketones and ethers.

2. Autoxidation. The centers where oxygen first attacks linolenic acid have been located by a combination of reductive and dehydration steps carried out on purified methyl linolenate hydroperoxide. The results showed that the terminal α-carbons of the polyene system have the highest reactivity towards oxygen. Knowledge of the preferential sites of oxidation is useful information in predicting the type and structure of compounds obtained on the decomposition of these hydroperoxides.

3. Removal of prooxidant metals. Studies on the use of ion-exchange resins to remove trace prooxidant metals from soybean oil are in progress under a PL 480 grant to the Institute of Fats and Their Derivatives, Seville, Spain. By means of statistically designed experimentation the effects of pertinent variables in removal of prooxidant metals with ion-exchange resins were evaluated. So far, the most favorable conditions appear to be treatment at 20° C. of 50 g. of soybean oil, diluted 1:1 with acetone, with 30 ml. of Amberlite CG-120, type I resin. By this procedure the following results were obtained:

	Metal content, p.p.m.[a/]			
	Fe	Mn	Cu	Zn
Original oil	1.19	0.255	0.085	1.23
"Demetallized" oil	0.23	0.000	0.017	0.00

a/ Determined by spectrographic analysis.

4. Effects of sterols on flavor stability. Initial experiments conducted at Gdansk Polytechnic, Gdansk, Poland, showed that autoxidation of soybean oil resulted in formation of oxidized sterol components.

B. Edible Utilization

1. Selective hydrogenation. Countercurrent distribution (CCD) was used after large-scale fractional crystallization to isolate and identify isomers formed in catalytic reduction of methyl linolenate. Selective combination of fractions from CCD yielded samples containing 95 percent cis,cis dienes, 90 percent mixed cis,trans and trans,cis dienes, 95 percent cis monoene, and 90 percent trans monoene. Analysis

of the diene fractions indicate that (1) the major cis,cis dienes have their bonds in the same positions as the original methyl linolenate, i.e., 9,12; 9,15; and 12,15; (2) bond migration took place during formation of the cis,trans and trans,trans dienes and of a small fraction of the cis,cis dienes.

CCD with a hydrocarbon-acetonitrile solvent (650 transfer stages) resolves a hydrogenation mixture on the basis of amounts of unsaturation present. This system, however, does not separate the isomeric monoenes and dienes. Gas chromatography (GLC) on a 200-foot capillary column packed with Apiezon L does fractionate the isomeric components after prior separation into monoene and diene classes. These two forms of differential migration (CCD and GLC) were applied to the products of catalytic hydrogenation of methyl and glyceryl linolenate to learn if steric effects influence the complexities of the reaction and its products. Great complexity was effectively demonstrated, but little difference was found except for a relatively faster reaction rate for monoester. Kinetic evidence for a "triene-to-monoene shunt" in catalytic hydrogenation has been obtained with the use of radioactive tracers. Preferential attack of the trienoic ester as compared to the dienoate ester was also shown. Chemical reduction of linolenate with hydrazine was studied with radioactive tracers, but no evidence for the anomalous shunt behavior was obtained. The reaction rates for the chemical reduction of triene, diene, and monoene appear to be in approximately the same ratio as the number of double bonds present.

Another difficult problem is separation of mixtures of geometric isomers of C_{18} fatty acids. Separation of cis,cis, cis,trans, and trans,trans isomers has now been achieved by preparing the silver π-complex followed by countercurrent distribution. Partially hydrogenated methyl linolenate separated by this method showed that cis monoenes largely retained the double bond at the original 9, 12, and 15 positions; and the trans monoenes contained widely distributed double bonds between the C_7 and C_{16} positions, demonstrating migration along with isomerization. This argentation technique was applied to a shortening and a hydrogenated winterized oil, both commercially prepared from soybean oil. The results showed that there was 24.6 percent of oleic acid and 27-28 percent of linoleic acid (cis,cis diene) in the winterized oil as compared to 17.2 percent and 9 percent, respectively, in the shortening. Trans content of the shortening was more than twice that of the winterized oil.

A new "iron reversion test" may simplify evaluation of partially hydrogenated or fractionated soybean oils for stability as salad and cooking oils. Soybean oil showed a rapid, large drop in the flavor score when 0.3 p.p.m. of iron was added, whereas cottonseed oil showed only a small drop. In contrast, soybean oil partially hydrogenated to less than 3 percent linolenate showed a flavor score equivalent to cottonseed oil by this test. Exposure to a controlled light source for

several hours also gave a large drop in flavor score for soybean oil but much less of a drop for cottonseed or hydrogenated, winterized soybean oil.

Commercial hydrogenation catalysts have been surveyed for selectivity and isomerization. Selectivity was determined by the "crossover point procedure" on a linoleate-linolenate equimixture under standardized conditions. Ratios of reaction rate constants for nickel catalysts at 140° C. ranged between 1.48 and 2.71; for palladium catalysts at 25° C., 1.68 to 2.50; and for platinum catalysts at 25° C., 1.33 to 1.61. Trans contents for these three metal catalysts ranged from 18.0 to 22.8 percent, 16.7 to 25.3 percent, and 7.5 to 8.4 percent, respectively. A study of the variables affecting selectivity of a single nickel catalyst showed that trans content varied from 45.9 percent at 230° C. to 14.95 percent at 70° C. with linolenic acid. No selectivity for the 15-16 double bond in linolenate was found. No commercial hydrogenation catalyst was found that gave the high selectivity required without formation of trans isomers. Whereas platinum produced the lowest trans isomers, selectivities were also low. Sulfur-poisoned nickel gave highest selectivity but also highest trans content. The demonstration of such variability gives hope that a catalyst or conditions of hydrogenation can be found which will give desired high selectivity and low trans.

Contract research at Armour Research Foundation showed that no truly selective catalyst is available for hydrogenation of the linolenic acid component of soybeans without producing appreciable amounts of saturated products or trans isomers. Catalysts included special products imported from Japan that were claimed to have desired selectivity but did not. Selective catalysts for linolenate reduction also produced high trans isomers. The more active catalysts had greater selectivity. Differences in formation of trans isomers and in activity were found, suggesting that the preparation of new catalysts might be a fruitful area to explore.

C. Industrial Utilization

1. Oxidative cleavage of soybean oil and its fatty acids. An important advance was the discovery that partial ozonization of soybean oil can be successfully accomplished. By partial ozonization, aldehyde oils that have controlled content of carbonyl groups, and therefore, controlled reactivity towards other chemicals can be readily prepared. It was also possible to prepare partially ozonized oils retaining various amounts of the original olefinic unsaturation, thus adding possibilities of thermal or oxidative polymerization to reactions characteristic of the carbonyl groups.

Since cost of ozone is the most significant factor in determining the cost of an aldehyde oil, partial ozonization enables important savings to be made. The following table shows "raw material" costs for several aldehyde oil products derived from different sources. This table shows that soybean oil (at nine cents per pound) would be preferred over tall oil (at nine cents per pound) for making aldehyde oils. Tall oil would have a slight advantage over soybean oil for making methyl azelaaldehydate, but soap stock (at five cents per pound) would be an economical source.

Product	Source			
	Margarine oil (9.5¢/lb.)	Soybean oil (9¢/lb.)	Acidulated soap stock (5¢/lb.)	Tall oil fatty acids (9¢/lb.)
	Cost, cents per lb.			
Aldehyde oil, 100% ozonized	18.9	20.3	--	27.8
Aldehyde oil, 33-1/3% ozonized	--	12.1	--	--
Methyl azelaaldehydate	--	22.8	20.0	22.6

The impurity formed in amounts in excess of 15 percent during catalytic hydrogenation of the ozonolysis products obtained from methyl oleate was found to be dimethyl azelaate. This impurity could not be separated from methyl azelaaldehydate or its dimethyl acetal by fractionation through a spinning band column. Pretreatment of the 10-percent palladium-on-charcoal catalyst with hydrogen did not prevent this oxidative degradation of the ozonolysis products. However, use of pyridine-methanol solvent substantially reduced but did not eliminate this undesired side reaction.

Pilot-plant ozonization equipment has been installed and used successfully for preparation of aldehyde oils, methyl azelaaldehydate and related materials.

2. Aldehyde oils and derivatives. Polyesterification studies of the bis (methyl azelaaldehydate) acetal of pentaerythritol with dimethyl terephthalate and ethylene glycol were continued. Sodium carbonate was found to be equivalent to lime as a catalyst in giving soluble, linear polymers. Crosslinking of these polymers was accomplished with p-toluene sulfonic acid, antimony trioxide, and magnesium oxide as well as those catalysts reported earlier. Crosslinking of poly(ester-acetals) has now been effected at temperatures of 100-150° C.

The use of the bis (methyl azelaaldehydate) acetal of pentaerythritol with dimethyl azelaate and hexamethylene diamine for polyamide formation was also explored. Crosslinking of the linear, soluble polyamides, which melt at over 230° C. and have molecular weights of 2,000 to 6,000, was accomplished by heating with p-toluene sulfonic acid. However, the amphoteric oxides that were effective crosslinking agents for polyesters were found to require higher temperatures to crosslink polyamides. The crosslinked polyamides showed strong adhesion to glass as did the crosslinked polyesters.

Studies conducted at the Eastern Division on the plasticizing action of various ester-acetal derivatives indicated that some of them were equivalent to or better than dioctyl phthalate as a plasticizer for polyvinyl chloride. New glyceryl derivatives of azelaaldehydic acid have been prepared and are being evaluated as components of polymers. Products obtained by partial ozonolysis of methyl linoleate and linolenate are now available for characterization.

Potential markets for soybean aldehyde products were estimated by Battelle Memorial Institute, in connection with its contract research on evaluation of these products, at 10 million pounds per year for aldehyde oils and 10-50 million pounds per year for methyl azelaaldehydate. Products prepared at Battelle that justify further evaluation include films obtained by the reaction of polyvinyl alcohol and methyl azelaaldehydate (monoaldehyde oil did not react), surface-active sorbitol and methyl glucoside derivatives, and factice-like products from diamines and dialdehyde oil. Studies comparing the use of free aldehydes and acetals indicated that acetals will usually give superior yields and products.

3. Separation of pure fatty acids. Research to determine the practicability of separating pure fatty acids from soybean and linseed oils is being conducted under a PL 480 grant at the University of Helsinki, Finland. By fractional crystallization without solvents it was possible to obtain considerable changes in the amounts of saturated fatty acids, but changes in the unsaturated ones were not significant. With small amounts of solvents saturated acids could be separated almost completely, oleic acid could be partially separated from linoleic and linolenic acids, but separation of the latter two acids did not appear to be feasible. Results reported to this time have not been encouraging for a solution to the problem of separating linoleic and linolenic acids from soybean and linseed fatty acids by a practical crystallization process. Current studies with zone refining using improved temperature control equipment may be productive.

4. New derivatives. At Queen Mary College, University of London, England, research is in progress on exploration of alkali fusion of unsaturated fatty acids and their derivatives to obtain new, potentially valuable derivatives of soybean and linseed oils. Alkali

fusion of both cis and trans 9,10-epoxystearic acid gave a mixture of mono- and dicarboxylic acids in 60-65-percent yield. Initial opening of the epoxide ring occurred in three ways: hydrolysis, elimination, and reduction. Attempts to arrest alkali fusion of oleic acid at the α,β-unsaturated isomer stage have, so far, resulted in detection of less than 5 percent of the desired product. Addition of a hydride ion donor, butyrolactone, in the alkali fusion of ricinelaidic and oleic acids, led to increased yields of myristic acid. With oleic acid and butyrolactone, both stearic and myristic were formed in amounts equal to 25 percent each of the usual yield for palmitic acid. Palmitic acid is not an intermediate. Effects similar to that of butyrolactone were achieved by use of a small excess of potassium hydroxide.

Research has been initiated at the Experiment Station for the Fats and Oils Industry, Milan, Italy, on studies of the effects of metallic catalysts and physical conditions on the reaction of atmospheric oxygen with soybean and linseed oils and their fatty acids and esters. This information will provide a basis for development of new industrial chemicals from these oils. Orienting experiments on catalysts, analytical methods and apparatus for conducting autoxidations have been performed. A method has been developed for detecting triglyceride peroxides with the aid of "chromatoplates" (silica on glass plates).

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AREA NO. 8: SOYBEANS
FEED, FOOD AND INDUSTRIAL USES FOR MEAL AND PROTEIN

Problem. Production of soybeans continues to increase rapidly and is expected to be about 700 million bushels in 1962. For profitable disposition, now and in the future, of the growing supplies of meal from U. S. soybeans, improved feed products and new food and industrial uses are needed. Europe is developing a mixed-feed industry that needs high-protein concentrates. This market could approach that in the U. S. which uses high-protein meal from 400 million bushels of soybeans. For U. S. soybeans to achieve the maximum share of this market, more fundamental information is needed on the proteins and other nutritionally important constituents of soybeans and on the effects of processing on these components. Such information should make possible the production of feeds from soybeans having maximum feeding value that would meet the requirements of foreign markets as well as help maintain or increase the use of soybean feeds in the U. S.

U. S. soybeans could play a dominant role in alleviating the world shortage of dietary protein if more information were available on utilizing soybeans and soybean meal, flour, protein and protein concentrates in food products tailored to meet the nutritional and palatability requirements of foreign markets. That the possibilities are very real for increased utilization of soybeans in foreign foods is indicated by recent work of the Department that showed how to use U. S. soybeans for Japanese foods. The result of this work was that a market for selected soybeans for Japan was opened that now exceeds one million bushels per year. If U. S. soybeans are to achieve the maximum share of foreign food markets, basic information on nutritionally important components and effects of processing on these components will be needed. In addition, better knowledge will be required of how to use soybean protein products in foodstuffs that will be acceptable abroad.

Opportunities also exist for developing new or improved products from soybean meal and protein for industrial use in adhesives, surfactants, emulsifiers, viscosity improvers, and related products. For example, a successful method for stabilizing soybean protein against microbial attack could result in regaining the market for soybean protein as viscosity improvers for water-base paints or as emulsifiers for asphalt. This potential could be realized if more basic information were available on the physical and chemical properties and chemical reactions of components of soybean meal.

USDA PROGRAM

The Department has a continuing long-range program involving organic and physical chemists and biochemists engaged in basic research on the characterization of components of soybean meal and protein and application of the knowledge gained to solution of problems encountered in processing and utilization of soybean meal and protein. This research is conducted at Peoria, Illinois.

The Federal scientific effort on utilization of soybeans and soybean meal and protein totals 8.0 professional man-years. Of this number 7.0 are devoted to chemical composition and physical properties and 1.0 to food products.

Research on chemical composition and physical properties involves basic studies on isolation and characterization of components of whey proteins and acid-precipitated proteins. Research on food products is devoted to development of information on specially processed protein products pertinent to their use in foods for foreign markets. The current program does not include research on industrial products. During the reporting period research on stabilization of soybean protein against microbial attack encountered in certain industrial uses was placed in abeyance to permit assignment of personnel to other important research.

The Department also sponsors research on utilization of soybeans (15.9 professional man-years) conducted by foreign institutions under grants of PL 480 funds. Research on chemical composition and physical properties (5.2 professional man-years) involves grants to the University of Edinburgh, Scotland, for investigations on polysaccharides of soybeans (3.2 professional man-years) and to the Weizmann Institute of Science, Rehovot, Israel, for research on complexes between soybean protein and other components of the meal (2.0 professional man-years). Research on food products (7.0 professional man-years) involves grants to the National Institute of Nutrition, Rome, Italy, for studies on use of soybean protein in pasta (3.0 professional man-years); and to the Central Miso Institute, Tokyo, Japan, for studies on miso made from dehulled soybean grits (3.0 professional man-years). Also, a contract, financed with PL 480 funds, has been placed with the Japan Shoyu Institute, Tokyo, Japan, for comparative evaluations of soy sauces prepared from Japanese and U. S. soybeans (1.0 professional man-year). Research on feed products (3.7 professional man-years) involves a grant to the Hebrew University, Rehovot, Israel, for basic studies on soybean saponins.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported 1.6 professional man-years divided among subheadings as follows: chemical composition and physical properties, 1.1; feed products, .1; and processing technology, .4.

Research dealing with the chemical and physical properties of soybean proteins involves separation and purification of the albumin and globulin proteins to establish amino acid sequence, molecular weight and peptide structure of individual purified proteins. Other stations are doing breeding and evaluation work with soybeans and a portion of this research includes testing and evaluating the meal and protein content of varieties. The program involving feed products deals largely with nutritive value considerations and the use of meal in animal rations. Research on processing technology centers around a process for the recovery of oil from oil-bearing seeds which is being developed. The process involves combination of the several unit operations of mixing, grinding, centrifugation, and decantation in a unique manner. Residual meals are evaluated.

Industry and other organizations maintain an interesting and vigorous program in this research area. Research on soybean meal and protein is conducted principally by soybean crushers and processors. Manufacturers of specialty foods and dietary products also contribute. The largest share of the companies' research effort is directed towards food products. Considerably smaller but approximately equal shares are devoted to basic research, processing research, research on feed products, and research on industrial products. Some cooperative work is performed in evaluation of processes and products developed by the Department. Except for such cooperative work, the exact nature of the research performed and the results are kept confidential or patented by the companies. Estimated annual expenditures by industry for research on soybean meal and protein products are equivalent to approximately 20 professional man-years in basic research, 40 professional man-years in applied and developmental research on food products, 15 professional man-years in research on feed products, 15 professional man-years in applied and developmental research on industrial products, and 20 professional man-years in processing research.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Acid-precipitated protein. The major component (11S protein) of acid-precipitated protein has been purified by ammonium sulfate precipitation. The starting material used is the cold-insoluble fraction of soybean protein which contains 70-75 percent 11S protein plus contaminants having sedimentation constants of 2S, 7S, and 15S. Preparations of 11S protein 91-96 percent pure have been obtained. Purified

11S protein exhibits a molecular weight of 350,000 at pH 7.6 and 0.5 ionic strength; it dissociates into 10 subunits at pH 2.2 and 0.01 ionic strength, and displays reversible aggregation to dimer plus small amounts of higher polymers when ionic strength is increased to 0.05. Freezing and thawing and freeze-drying also result in breakdown into subunits and reaggregation. The 11S protein does not, however, revert to the original distribution of components in the unfractionated mixture. In the course of this work it was noted that the ratio of 7S to 11S components varied with the type of soybean. Whether changes in this ratio are actually varietal or merely environmental has not yet been established.

2. Whey proteins. The removal and recovery of whey proteins by precipitation with anionic detergents and edible gums and partial evaluation of the properties of the precipitates have been completed. The results show that the whey proteins are completely precipitated by selected negative colloids, that essentially all the lipoxidase is present in the precipitate (complex) in an active form, but that an unsaturated fatty acid must be combined with the complex to obtain enzyme activity. Also the amylases were precipitated in an active form, but because of the insolubility of the complex at the pH of optimum activity, the extent of its activity was not fully established. The maximum decrease in the BOD of the whey solution was 18 percent. This procedure may lead to development of commercially feasible processes (1) to isolate active enzyme preparations from the whey and (2) to reduce the manufacturing cost of soybean protein.

By means of improved chromatographic procedures, two trypsin inhibitors (A_1 and A_2) were isolated from whey protein in 96-percent purity. They were compared with commercial Kunitz (K) inhibitor of at least 95-percent purity. Molecular weights of A_1, A_2, and K determined in the ultracentrifuge were 14,000, 21,600 and 22,700. These and other physical data indicate that A_1 is different from A_2 and suggest that A_2 and K are the same. The specific activities toward trypsin for the three are in the ratio 1.6:1.0:1.0; however, the molar ratios are 1.0:1.0:1.0, showing a mole-for-mole reaction for each of the three preparations with trypsin. On the other hand, preliminary amino acid assays of A_2 and K show that inhibitor K is significantly higher in isoleucine, glycine, valine, tryptophane, and glutamic acid content. The N-terminal residue for all three inhibitors is aspartic acid. Preparation A_1 is an entirely new trypsin inhibitor. Further study will be needed to determine if compositional differences between A_2 and K are a matter of impurities or of identity.

Factors responsible for growth inhibition and pancreatic hypertrophy of rats fed raw soybean meal were readily destroyed by autoclaving for 15 minutes at atmospheric pressure and 100° C. Assay by growth rate of rats was maximum even with 120 minutes of autoclaving at one atmospheric pressure with live steam. Initial moisture of 5 and 20

percent had no effect. These results are not in agreement with previous work that showed 30 minutes autoclaving at atmospheric pressure was required for maximum growth and that there was a decline in growth with heating beyond 60 minutes. Our results explain the high degree of uniformity in nutritive value of eight commercial meals obtained from five processors even though processing conditions of temperature, time and moisture varied considerably. Assay of soybean meal fractions, residue, acid-precipitated protein, whey solids, and isolated whey proteins showed that autoclaving was required for maximum growth rate and to prevent pancreatic hypertrophy. Factors responsible for growth inhibition and pancreatic hypertrophy are concentrated in the whey fractions. Growth inhibition and pancreatic hypertrophy with rats fed raw soybean meal can be reversed by switching to casein. Little correlation was found between trypsin inhibitor activity of meal fractions and pancreatic hypertrophy. Poor protein efficiency of isolated protein is corrected by adding methionine. In connection with this work the complete amino acid analyses of soybean meal fractions were determined for the first time. Three of these fractions are commercial byproducts, production of which total at least 400,000 tons annually. Animal feeding tests were conducted in the Pharmacology Laboratory of the Western Division.

3. Soybean polysaccharides. Research at the University of Edinburgh, Scotland, has resulted in the isolation of a pure galactomannan consisting of a main chain of mannose units with galactose attached to every third mannose unit.

B. Food Products

1. Reversible gels from soybean protein. It was discovered that ethanol washing of the acid-precipitated protein enhances its foaming properties. Further work showed that alcohol washing removes an "antifoaming agent(s)" and that alcohol denaturation of the protein is not responsible for the increased foaming properties. Removal of the antifoaming agent by alcohol washing was markedly dependent upon alcohol concentration; 86 percent aqueous concentration was most efficient for ethanol. Acid-precipitated protein washed with this concentration of alcohol can be whipped into a foam which is 30-40 times as stable as the foam from unwashed protein or commercial soybean whipping agents. Heat-reversible gels form when 6-10 percent neutral solutions of alcohol-washed protein are heated and then cooled. Studies suggest antifoaming and gelling agent(s) is(are) phospholipide(s). Aqueous methanol (95 percent) and aqueous isopropanol (82 percent) were as effective as 86 percent ethanol for washing acid-precipitated soybean protein to improve foaming properties of the protein.

Development of unusually stable foams or heat-reversible gels from soybean protein may lead to increased industrial and food use of this protein; for example, these foams may prove important in foam mat

drying. Evaluation tests of these foams in drying orange juice are in progress at the U. S. Fruit and Vegetable Products Laboratory, Winter Haven, Florida.

2. Comparison of United States and Japanese soybeans for soy sauce. Quantitative data have been reported on the preparation of soybeans, wheat koji and other steps leading to the fermenting stage in manufacture of soy sauce at 11 different processing plants. Significant results on yields and quality of shoyu cannot be given until after the fermenting stage which will last for several months. This research is being conducted by the Japan Shoyu Institute, Tokyo, Japan.

C. Industrial Products

1. Stabilization of soybean meal fractions against microbial attack. In continuation of studies to increase industrial utilization of soybean meal and protein by stabilization to attack by microorganisms, the following chemicals were reacted with defatted soybean meal under one or more sets of conditions: (1) carbon disulfide, (2) ethylene oxide, (3) butylene oxide, (4) phenyl isocyanate, (5) maleic anhydride, (6) potassium thiocyanate, (7) phthalic anhydride, (8) acrylonitrile, and (9) glycidyl methacrylate. Similar reactions were effected on α-protein and sodium proteinate. Several of above reactions were effected on the residue from protein isolation and soybean whey proteins. Stability of the reaction products was tested by measuring the change in pH and viscosity of the reaction products and time for the development of putrefaction after inoculation with soil. Effect of addition of formaldehyde on viscosity was determined. None of the reaction products was stable to putrefaction more than 4 days after inoculation with soil. Products which showed the greatest stability to microorganisms and showed little or no change in viscosity of the protein on addition of formaldehyde were the reaction products of ethylene and butylene oxide, maleic and phthalic anhydride, phenyl isocyanate and acrylonitrile. The ethylene oxide reaction product with α-protein was very unusual in that it was soluble in the pH range of 1.8-12.0. Use of a chemical preservative, such as formaldehyde, after appropriate chemical modification of the protein has the greatest promise for industrial utilization where extended stabilization is important. This research has been placed in abeyance to permit assignment of personnel to more urgent work.

D. Feed Products

1. Effects of saponin on nutritional quality of soybean feeds and foods. A critical review of the literature has been made, methods for extracting saponins from soybeans have been devised, and assay methods for the quantitative determination of soy saponins have been established. Experiments show that autoclaving soybean meal destroys most of the

hemolytic activity of the saponins present, but autoclaving isolated saponin preparations had no effect on this biological response. These results indicate that cooking soy food and feed products may be important to inactivating the suspected "toxic" effects of saponins in these products. This research is being conducted by the Hebrew University, Rehovot, Israel.

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AREA NO. 9: REPLACEMENT CROPS UTILIZATION POTENTIAL

Problem. Farmers could achieve more economic use of their land if new and profitable crops were available for their choice that would have different end-use patterns from those presently grown. For example, it would be advantageous to develop a new oilseed crop yielding unique fatty acids that could find industrial use in applications for which acids from presently available domestic oilseed crops are unsuitable. To develop a new crop, three basic steps are involved: (1) survey of wild plants, in cooperation with plant scientists, to identify those having both potentially valuable components and promising agronomic potential for use in the United States; (2) detailed physical and chemical characterization of components of interest to obtain clues to likely end uses; (3) selection of the most promising species followed by additional utilization research to explore uses and demonstrate industrial potential and by additional agronomic research to establish proper cultural practices and to select the best strains and varieties. Only after these steps have been successfully accomplished can a proposed new crop be offered to agriculture and industry for introduction and development. Obviously, a program of this type is a long-range one. Yet, whether the future of agriculture involves conditions of surplus, of greater emphasis on foods and feeds, or of necessity for greater national self-sufficiency, the nation will benefit from availability of optimum, practical crop plants to serve its needs.

To achieve the objective, survey and characterization work needs to be greatly increased, since the greater the number of species examined, the greater will be the opportunities for finding plants meeting the criteria of high utilization and agronomic potentials. Work of the Department has already revealed several promising sources of new potentially valuable water-soluble gums, pulp fibers, and oils containing unique fatty acids such as hydroxy-unsaturated acids, capric acid, epoxidized acids, and unusual long-chain fatty acids. In order to demonstrate the potential of these new materials, further work is required on their physical and chemical properties and reactions, on processing to obtain maximum recovery from source plants, and on by-products from processing, such as oilseed meals.

USDA PROGRAM

The Department conducts a long-range continuing program of research involving analytical and organic chemists and chemical engineers engaged in examination of uncultivated plants to find unusual and potentially useful components and in detailed characterization and evaluation studies of selected components that have the greatest

industrial potential and that are obtainable from agronomically promising plants. Plants or seeds for this program are obtained by cooperation with Crops Research Division which procures material from domestic and foreign sources by means of collecting trips or from experimental plantings. Materials from abroad are also made available through Crops Research Division PL 480 projects providing for collecting activities by foreign investigators. All seeds and plants are submitted to a broad chemical-screening program to identify sources of unusual and potentially useful components such as oils, fibers, gums, amino acids and proteins. Components of interest from plants rated by Crops Research Division as having a reasonable agronomic potential for the United States are characterized to obtain clues to areas of utilization of probable interest to industry. On the basis of the results, plants having the highest agronomic potential and containing components of greatest potential industrial value are selected for more intensive utilization research. This utilization research is divided among the four Utilization Research and Development Divisions.

The Federal scientific effort devoted to research on replacement crops at Peoria, Illinois, totals 19.7 professional man-years. Of this number, 11.7 are concerned with chemical composition and physical properties; 6.2 with industrial utilization of new oilseeds; and 1.8 with industrial utilization of new gum and fiber plants.

Research at Peoria, Illinois, on chemical composition and physical properties (19.4 professional man-years) involves conduct of the program on screening uncultivated plants for unusual and potentially useful oils, fibers, gums, amino acids and other components; organic chemical characterization of selected fractions and components, especially new oils fatty acids; and studies on properties of new plant fibers. During the reporting period, specific projects on screening for proteins were discontinued because this work can be conducted more effectively as part of the screening programs for new oilseeds and amino acids. A research contract (.3 professional man-year) is in effect with Montana State College, Bozeman, Montana, providing for screening and analysis of seed oils of Brassica (mustard) and related genera to identify species having greatest erucic acid content and agronomic potential.

Research at Peoria, Illinois, on industrial utilization of new oilseeds (6.2 professional man-years) involves studies on processing of erucic acid oilseeds to obtain oil and meal and investigations on utilization of erucic acid and its derivatives.

Research at Peoria, Illinois, on industrial utilization of new gum and fiber plants (1.8 professional man-years) is concerned with development of methods for recovery of gums from plants; with evaluation of plant gums in industrial application; and with studies on pulping new

fiber plants and evaluation of the pulp in paper, structural boards and related products. During the reporting period research on utilization of Crotalaria intermedia gum was completed.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 3.7 professional man-years divided between subheadings as follows: chemical composition and physical properties, 2.2; industrial utilization, 1.5. The Hawaii Station is making assays of the native and introduced tropical plants in a search for products of economic value. Other station research involves screening studies of the chemical composition of many domestic and exotic plants. A study which is cooperative with USDA is seeking ways to modify or improve methods of retting fiber crops. Methods are sought which will yield maximum fiber of high, uniform quality. Industrial utilization of replacement crops is the subject of effort devoted to determining possibilities for industrial use for a number of domestic and imported crops. High oil-producing crops, which produce oils of specific character, are actively being sought and their potential evaluated.

Industry and other organizations conduct significant research on replacement crops. Several industrial companies are interested in the utilization of materials derived from foreign or potential new domestic crops for solving special problems encountered in their operations. Some companies sponsor experimental plantings in the United States. Others are not interested in possible domestic production of crops but intend to produce and use materials in foreign operations. Still others investigate materials from foreign crops for use within the United States. Commodities of interest include plant gums, new pulp fibers, and oilseeds containing unusual oils and fatty acids. The companies' research in these areas is largely devoted to process and product development and evaluation studies. Some effort is placed on basic research, especially on plant gums. A good share of the industrial research effort is concerned with cooperative evaluation and testing of products now being studied for utilization potential by the Department. Except for cooperative work, the exact character and results of the companies' research is kept confidential or patented.

A small research effort related to utilization potential of replacement crops is expended by universities. Basic research is concerned with development of methodology for analysis of certain unusual fatty acids and with study of the effects of these acids in animal feeding. Applied research involves study of utilization of an oil from a new oilseed in protective coatings. Estimated annual expenditures for research on replacement crops are equivalent to approximately 7.0 professional man-years in basic research, 10.0 professional man-years in applied and developmental research on new pulp fibers, 15.0 professional man-years in applied and developmental research on plant gums, and 6.0 professional

man-years in applied and developmental research on unusual oils and fatty acids.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Screening for new industrial oils. Since the last report 1,087 additional samples of seeds were screened for new oils of potential industrial interest. Lesquerellae. All but two of fourteen species tested produced mainly the C-20 hydroxy acid (lesquerolic acid) analogous to ricinoleic acid. The two exceptional species produced a C-18 hydroxy acid. Crop potential of Lesquerellae is good. Industrial possibilities for utilization of lesquerolic acid appear attractive; e.g., this acid can be readily converted to dodecanedioic acid, a C-12 dibasic acid that merits evaluation as an intermediate for resins, plasticizers and lubricants.

Cupheae. Two species of Cuphea contained over 80 percent of capric acid. A third species contained 57 percent of lauric acid (compare 44-52 percent for coconut oil). Large quantities (over 600 million pounds) of coconut oil are imported each year because of its content of lauric, capric and other acids having fewer than 18 carbon atoms in their chains; hence a domestic source of such acids would be valuable. Unfortunately crop potential of the Cuphea genus has not proven to be promising. Hopefully, continued screening may reveal other genera rich in short chain acids.

Erucic acid oils. Some 270 seed samples of Cruciferae have so far been analyzed during the contract research at Montana State College. Two samples of Brassica chinensis (Chinese cabbage; good crop potential) seed showed 48-50 percent of oil and 21-22 percent of protein. The oil contained 48-51 percent of erucic acid. However, no species or variety has been encountered that contains as much or more erucic acid in its oil than Crambe abyssinica (28-36 percent oil containing 56-59 percent erucic acid; 23-29 percent protein).

Miscellaneous unusual oils. Many seed oils containing unusual fatty acids or other uncommon components were revealed by the screening program. Space permits mention of only a few of the more interesting results. The following examples will, however, serve to illustrate the remarkable variety of constituents observed in uncultivated oilseeds. Several Cruciferae contained up to 58 percent of eicosenoic acid, a C-20 monoene acid. An aster oil contained trans unsaturation and 12 percent of a C-16 monoene acid (apparently not palmitoleic acid). Comandra pallida seed oil contained 45 percent of ximenynic acid. This is the first domestic source observed to contain substantial amounts of an acetylenic acid. Oil from Osyris alba (sandalwood family, Turkey)

contained 59 percent of ximenynic acid. A source containing 36 percent of an unidentified triene acid having trans unsaturation, previously observed in several aster species and burdock, was found in Calea urticae-folia (sunflower family). This acid is different from the triene acid of Thalictrum.

Seed oil of Melochia charantia (from Mexico) contained 50 percent of conjugated triene. Tetraenoic acids were observed in oil from Lappula redowski and in several other borage oils. Dalea oils contained 28 percent of unsaponifiables high in xanthophyll. The lipid extract from seed of Briza spicata, a Turkish grass, was a greasy solid that did not melt on the steam bath. It contained 1.6 percent of nitrogen and 3.0 percent of phosphorus but only 50 percent of fatty acids. Tests also showed presence of major amounts of sugars.

2. Studies on oilseed meals. The economic value of any new oilseed crop would be greatly enhanced if it yielded a nutritious, high-protein meal for animal feed. During the reporting period special attention was paid to minor constituents that might be deleterious to feeding value, especially isothiocyanates and oxazolidenethiones of crucifers. ("Oxazolidenethiones" are sometimes less precisely named "thiooxazolidones.") Seven samples (four locations) of Crambe abyssinica grown by the NC-7 Committee (North Central Regional Committee on New Crops) produced extracted meals containing from 1.7 to 2.5 mg. of isothiocyanate (calculated as butenyl) per gram of meal (seeds not hulled) and 3.0 to 5.5 mg. of oxazolidenethione. Seven samples of Eruca sativa from the same locations contained from 18.2 to 19.9 mg. isothiocyanate (as butenyl) per gram of extracted meal except for one (probably immature) sample that contained 11.1 mg. E. sativa contained no oxazolidenethione. Extracted meal from two samples of Lesquerella lasiocarpa contained 6.9 and 7.4 mg. of volatile isothiocyanate, while three other species ranged from 2.4 to 3.9 mg./g. No oxazolidenethione was found in the Lesquerella seed examined.

Certain heat-processed seed meals from Brassica juncea showed considerable losses in lysine and in some cases histidine and arginine, indicating the need for care in establishing heating conditions. Several unfamiliar amino acids were indicated in meals from Acacia willardiana, Acacia farnesiana, and Lysiloma desmostachys. Meal from Sesbania macrocarpa showed some promise as feed material, even when fed raw to rats and mice; that from Limnanthes douglasii maintained life, but permitted little growth; and Clitoria ternatea meal appeared to be toxic. Best results with all three meals were obtained after heating. The poor results with L. douglasii may be attributable to bulkiness or other factors rather than to protein quality. Further testing is in progress on L. douglasii and S. macrocarpa. Studies have begun to investigate possible solvent systems for removal of thioglucosides

from Crambe and to develop improved analytical methods for thioglucosides. Lunaria annua meal prepared by the Northern Division detoxification process was nontoxic and nutritious in feeding tests with rats at the Western Division.

3. Screening for amino acids. Evaluation was made of results of amino acid analysis on seeds of 200 plant species in 181 genera in 61 families. Previous indications were confirmed that the amino acids showing greatest variability, other than hydroxyproline, were lysine, methionine, arginine, glutamic acid, and proline. Leguminosae tend to be high in lysine and low in methionine; Compositae, low in lysine and high in methionine; and Cruciferae, high in lysine and average in methionine. A mean of 85.6 percent of the nitrogen was accounted for as common amino acids and ammonia and 3.4 percent in insoluble humin leaving 11 percent unidentified or undetected. Additional determinations of amino acid composition revealed a seed meal from one species, Cephalotaxus harringtonii, that contained 31 percent protein high in lysine (6.6 g./16 g. N), methionine (2.3 g./16 g. N), and four additional essential amino acids. This species has, however, little potential for crop development.

Further studies were made of the hydroxyproline content of seed meals, seedcoats, and pericarps. Hydroxyproline was found in 123 of 183 seed meals analyzed, to the extent of 0.1 to 5.7 g./16 g. N. Eight samples of seedcoat and pericarp contained from 3.1 to 10.0 g./16 g. N. In 16 seed meals derived from kernel alone there was no hydroxyproline. Solubility studies indicated that hydroxyproline was a part of plant protein, an observation of fundamental importance since this amino acid had hitherto not been considered to be a constituent of normal plant protein.

4. Screening for new seed mucilages. All species of Crotalaria available at the Northern Division have been surveyed for mucilage content. None was superior in yield to C. intermedia except C. spectabilis which has two disadvantages--dark color and extreme toxicity. Thirty-seven new species of seed have been surveyed for mucilage content. Two Cassia species containing 29 to 32 percent water-soluble mucilage in the seed are reported to produce 1,837 pounds per acre (C. occidentalis) and 2,875 pounds of seed per acre (C. bonariensis).

5. Screening for new pulp fiber plants. One-pound pulping experiments were performed on 61 raw materials to investigate the relationships among compositional and botanical characteristics and pulping properties. Data were collected on pulp yield, chemical consumption, permanganate number, ease of refining, pulp freeness, and strength values of handsheets from the pulps. Statistical evaluation resulted in a number of significant and interesting conclusions. A few of

these are: (a) Given equivalent kraft pulping treatments, grasses are more completely pulped than dicotyledons as indicated by extent of delignification. (b) Pulps from grasses contain more pentosan, primarily because of larger amounts present in the starting materials; percentage removal on pulping is the same as with dicotyledons. (c) Differences in alkali solubility are considerably more statistically significant than are differences in cellulose content when plant groups are compared with one another; alkali solubilities parallel differences in pulp yield. (d) Plants of the mallow family yield pulps that refine easily and form paper with high fold and burst values. (e) Fiber dimensions are not as closely associated with sheet strengths as expected. (f) Within plant families (contrast with point c) α-cellulose content is the property that would serve as the best single criterion for predicting both pulp yield and pulp quality for a given species. One species each of Sesbania, Crotalaria, Hibiscus, and Andropogon, as well as a new okra variety, had superior pulping possibilities in comparison with other new samples screened.

6. Characterization of new seed oils and components. Lunaria biennis seed oil was shown to contain 21 percent of cis-15-tetracosenoic acid (C-24) in addition to 42 percent of erucic acid. This oil might be a practical source of the C-24 acid since it is readily separated from erucic acid by distillation. Hibiscus syriacus seed oil was found to contain over 16 percent of malvalic acid plus 3.4 percent of sterculic acid (C-18 and C-19 cyclopropenoid acids, respectively). This species is the richest herbaceous source of cyclopropenoid acids yet found. In addition, both malvalic and sterculic acids were found in several species thought to contain only one of these acids. These acids are important because they are reactive acids that should have high industrial potential if good sources can be found. They also have important physiological properties when found in trace quantities in poultry feed.

The triene acid in Thalictrum polycarpum seed oil (35 percent of acids) has been identified as trans-5,cis-9,cis-12-octadecatrienoic acid. The "hydroxy" acid in Ipomoea sp. seed oil is (+)-11-hydroxyhexadecanoic acid. The new hydroxy fatty acid in Lesquerella densipila oil (35 percent of the seed oil) has been characterized as 12-hydroxy-cis-9,cis-15-octadecadienoic acid. The discovery of a naturally occurring long-chain acid having hydroxyl function located between two double bonds and constituting a major component of a seed oil again extends the range of raw materials that can be obtained from higher plants.

The glycolipid component of Ipomoea parasitica seed oil is an acylated glycoside of (+)-11-hydroxypalmitic acid which on the average contains two sugar (monosaccharide) units per molecule. Physical and chemical properties have been determined for oils; fatty acids, alcohols and methyl esters; liquid and solid wax esters; and hydrogenated triglycerides from Crambe abyssinica and Lunaria annua (biennis). The wax esters were recovered in yields of about 80 percent of theory based on oil.

B. Industrial Utilization of New Oilseeds

1. Processing mustard and other erucic acid oilseeds. By a suitable sequence of operations it has been found possible to integrate an efficient detoxification step into the conventional filtration-extraction method for recovering oil and meal from oilseeds. After cracking and flaking, mustard seed was moisturized and held at a moderate temperature to permit natural enzymatic hydrolysis of the glucoside to liberate allyl isothiocyanate. The wet meal was then heated and cooked with simultaneous removal of the allyl isothiocyanate by distillation. After cooling, the crisped meal was re-rolled, and submitted to filtration-extraction. Remaining traces of allyl isothiocyanate were removed from recovered oil by conventional refining techniques and from the meal by a final treatment with live steam. With cooperation of the Southern Division large batches (up to 500 pounds) of mustard seed were processed in the pilot plant by the process described. Whereas the original seed contained 0.92 percent of allyl isothiocyanate, the detoxified meal contained 0.004 to 0.007 percent and the refined oil contained no detectable allyl isothiocyanate.

With the initially developed process just described, recovery of allyl isothiocyanate and lysine content of the meal were unsatisfactory. These problems have been substantially overcome by reducing the enzyme hydrolysis period (liberation of allyl isothiocyanate from its glycoside), sparging with steam to remove the volatile oil, and reducing the overall heating period. Rat-feeding tests conducted at the Western Division with mustard meal produced by the Northern Division integrated process showed that the mustard meal was nontoxic and was utilized quite well, although not quite equal to commercial soybean meal. Thyroid tissues taken in autopsies showed no evidence of enlargement even when the animals had been fed a ration containing 0.4 percent of allyl isothiocyanate. In laboratory trials detoxification has also been integrated successfully with the prepress-solvent extraction process.

Processing studies were initiated on Crambe abyssinica seed to test the integrated filtration-extraction procedure on this species. Meals were obtained that contained no detectable oxazolidenethione and little (0.005 percent) allyl isothiocyanate. The apparent elimination of oxazolidenethione in Crambe meal should be regarded with considerable caution. The mechanism and products of decomposition of oxazolidenethione during processing are not known. Absence of oxazolidenethione does not necessarily signify absence of toxicity. A supply (10 pounds) of Crambe meal, shown by presently available analytical techniques to contain no oxazolidenethione and negligible isothiocyanate, has been sent to the Western Division for feeding tests. Except for completion of cost analysis, study of processing of ordinary mustard seed is essentially complete. Emphasis will now be placed on Crambe processing and the resulting fate of oxazolidenethione.

2. Utilization of erucic acid. Erucic acid of 90-percent purity was produced from fatty acids prepared from Crambe abyssinica oil (50 percent erucic acid) by liquid-liquid extraction, followed by distillation. Ozonization of methyl erucate and reductive cleavage of the ozonides gave a 75-percent yield of methyl brassylaldehydate of 95-percent purity. Similar procedures applied to mustard seed oil gave an 88-percent yield of an aldehyde oil containing 2.67 carbonyl groups per molecule. Workable procedures for preparing pound quantities of brassylic acid (97.5 percent pure) from erucic acid have been developed and samples have been submitted to the Western Division for evaluation in shrink-proofing of wool.

C. Industrial Utilization of New Gum and Fiber Plants

1. Crotalaria gum. A dry-milling procedure was developed for milling Crotalaria intermedia in which the whole seed is passed through a pin mill at 18,000 r.p.m. to shatter the hull and pulverize the germ, leaving the gum in the form of relatively coarse particles retained on a 40-mesh screen. The fines are separated from the coarse gum particles by air classification or by screening, or a combination of these. By flaking of the +40-mesh particles in a roller mill and grinding in a hammer mill, the gum is reduced to flour (-100-mesh screen) for use-testing. In color and viscosity of solutions, the recovered gum was similar to commercially available guar gum. A steaming procedure for enzyme inactivation was devised which prevents the rapid loss of viscosity of solutions of Crotalaria gum. Inclusion of a tempering step in the processing gave up to a twofold increase in viscosity of the solutions. This dry-milling process has also been utilized successfully for isolating gum from seeds of guar and Cassia marilandica (a legume). Yields of gum are 23.5 percent from Crotalaria and guar and 27.5 percent from Cassia. In preliminary tests as a wet-end additive for softwood kraft and sulfite paper pulps, Crotalaria gum gave results equal or superior to guar gum in improving burst, tensile strength, and fold properties of paper handsheets.

2. Kenaf for pulp and paper. Based on the results of screening tests of several hundred plant species as possible sources of nonwood pulps and on the evaluation of the agronomic potential of these plants by Crops Research Division botanists and agronomists, kenaf and related Hibiscus species were selected for more detailed evaluation of their industrial potential.

Yields of unbleached and bleached kenaf pulps (about 46 and 41 percent, respectively) from soda and kraft cooking (18 and 21 percent chemical) were comparable to yields of pulps from coniferous wood by the sulfite and sulfate processes. Yields by the neutral sulfite process (54 to 57 percent unbleached and 45 to 51 percent bleached) were substantially less than those for hardwood neutral sulfite semichemical pulps (75 percent unbleached and 58 percent bleached). Values for chemical

consumption in the soda and kraft processes were of the same magnitude (about 85 to 95 percent) as for sulfate pulping of Douglas fir at comparable levels of applied chemical. Chemical consumption by the neutral sulfite process amounted to 72 to 77 percent; however, these values are not directly comparable with available data on neutral sulfite pulping of woods.

Burst and tensile properties of handsheets from the kenaf pulps were substantially comparable to published data for sulfate pulps from Douglas fir. Tear strength of the experimental papers was deficient by comparison, but at a usable level. Data for fold strength approached rather closely the fold data for the Douglas fir pulps. Experimental machine papers from kenaf had strength properties, except for tear, comparable to those of published data for kraft bond papers from Douglas fir.

Kenaf pulp was blended at various levels with representative commercial soft- and hardwood pulps. In most cases properties were intermediate and as anticipated on the basis of percentage composition. Thus, kenaf-hardwood blends had superior properties to hardwood pulps alone. In blends of kenaf and softwood kraft pulps, folding endurance and bursting resistance were greater than expected. Folding endurance was somewhat less than predicted for kenaf blends with hardwood-sulfite and groundwood pulps, but tear and opacity were improved.

Work was initiated on development of mechanical-type pulps for structural board from kenaf and a few related species. Chopping of the original materials to closely controlled sizes ranging from 1/2 to 1 inch, or dry shredding of original materials in a disk mill prior to cooking, appears to alleviate the earlier problem of nonuniformity associated with bimodal fiber systems in pulps from dicotyledons. Freeness requirements have been met by disk milling of materials cooked in weak neutral sulfite or soda solutions; however, the experimental pulps were still coarse and not in the acceptable range of characteristics in comparison with a reference wood pulp known to be suitable for resin-bonded fiber hardboard.

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AREA NO. 10: SUGARCANE PROCESSING AND PRODUCTS

Problem. Quotas established by the Sugar Act effectively prevent the accumulation of surpluses by limiting production to estimated requirements at stable, low prices for sugar. Prices received by farmers of the United States and Puerto Rico for sugarcane are based upon the recoverable sugar content of the cane; and the rising costs of production and processing make imperative the more efficient recovery of increased amounts of sugar to provide adequate returns for both processors and growers. Currently recovery of 75 percent of the total sugar in the cane is considered satisfactory in Louisiana, and about 83 percent in Puerto Rico and Hawaii. Improved processing methods could increase the recoverable sugar to at least 85 percent in Louisiana and over 90 percent in other areas. The development of more efficient processing methods depends in turn upon the acquisition of adequate data on the quantitative composition of juices extracted from sugarcane, and of materials processed to recover sugar. The chemical industry provides a promising potential for the utilization of additional sugar since more than 15 billion pounds of chemical products are produced annually and sold to every section of American industry. More information is needed on the chemistry and properties of products from sugar to expand their utilization and on the application of these derivatives in the production of plastics, protective coatings, emulsifiers, detergents, and the like.

USDA PROGRAM

The major part of the Department's research program on sugarcane processing and products is maintained at the Southern Utilization Research and Development Division, New Orleans, Louisiana. At the Northern Division, Peoria, Illinois, the Department maintains a long-term continuing program requiring a part of the services of a biochemist and an analytical chemist engaged in basic and applied research on the fermentative conversion of sugar to industrially useful organic acids.

The Federal program at Peoria, Illinois, totals 1.0 professional man-years, all of which is devoted to new and improved products, specifically, fermentative conversion of sugar to α-ketoglutaric acid.

In addition, the Department, through the Northern Division, sponsors research under a grant of PL 480 funds to the Institute of Biological Chemistry, University of Rome, Italy, for studies on the preparation and characterization of dextran derivatives. This research totals 3.0 professional man-years, all of which is under the subheading, new and improved products.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

Related programs of State Experiment Stations and of industry and other organizations are reported by the Southern Utilization Research and Development Division in its "Summary of Current Program and Preliminary Report of Progress."

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. New and Improved Products

1. Production of α-ketoglutaric acid. Conversion of the intermediate acid, 2-ketogluconic (2KGl) is the rate-limiting step in α-ketoglutaric acid (αKG) production from sucrose. Accordingly, short-term oxidations of glucose, gluconic acid, and 2KGl with nongrowing cells were studied to determine what might be done to modify the rate of utilization of glucose, gluconic acid, and 2KGl. Results of this approach were disappointing because nongrowing cells of Pseudomonas reptilivora NRRL B-6bs did not convert any of the indicated substrates at usefully rapid rates.

Variation in the age of cells, medium used to grow the cells, suspension milieu, buffer type and concentration, or pH of the system failed to induce rapid cellular oxidations in absence of growth. Results based on the more sensitive dye reductions have shown that glucose and gluconate are oxidized 5-10 times faster than 2KGl and that no modifications have induced changes in oxidation of 2KGl different from those induced in oxidation of glucose and gluconate.

Glucose and 2KGl-grown (adapted) cells were tested for their ability to produce αKG from 2KGl as the substrate in conventional fermentations. Cells grown on 2KGl utilize 2KGl more rapidly than do glucose-grown cells but the subsequent rate of conversion and final yield of αKG are not different. Yields of αKG from 2KGl are exceedingly variable. In extreme cases CO_2 appears to be the only product in some 2KGl fermentations. Survey of 161 species or strains of bacteria revealed 12 strains of Pseudomonas equal, or somewhat superior, to P. reptilivora in ability to produce αKG from glucose. Two bacteria were found that produced no 2KGl; however, their ability to produce αKG was about the same as that of P. reptilivora. One species of Serratia appears to deserve more detailed study.

For fermentative production of αKG to be an economical and industrially practicable process, yields and production rates must be considerably increased above present levels. Further effort to achieve this goal is justified by the potential industrial value of this acid and the improbability of inexpensive synthesis from nonagricultural raw materials.

2. Studies on dextran derivatives. Probing experiments at the Institute of Biological Chemistry, University of Rome, Italy, have delineated the most suitable approaches to achieve the objectives of the project. Techniques and analytical procedures have been standardized.

PUBLICATIONS AND PATENTS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

None.

AREA NO. 11: FORAGES AND FEED PROCESSING AND PRODUCTS

Problem. Fresh forage crops are the richest natural sources of a wide variety of nutrients essential to farm animals. The bulk of these crops, however, is preserved by such inefficient processes (hay making and ensiling) that 10 to 50 percent of the original dry weight and much larger amounts of certain valuable nutrients and growth-promoting factors are lost before the animal consumes the products. Dehydration is currently the only practical means for preserving a high percentage of the value of forage crops. Poultry and swine producers, aware of the value of dehydrated forage in feeds, nevertheless restrict the use of this product because of its high fiber and growth-inhibitor content. The livestock producer needs, and therefore the forage dehydrator needs to produce, feed ingredients from forages, tailored to specific classes of farm animals. Intensive basic and applied utilization research are needed to develop new methods for processing forages to produce: (1) high-value, fiber-free juice or low-fiber products for nonruminant animals; (2) low-cost products, rich in fiber treated to make it highly digestible, for ruminants; and (3) a growth-stimulating supplement for ruminants, taking advantage of the presence in forages of such growth-promoting compounds as the recently discovered coumestrol. Forage products for ruminant feeding would be specifically designed for the mechanized feeding operations which will be essential for the 45-percent increase in livestock production to meet the projected 1975 requirements. Development of new processes and improved forage products would stimulate the production of large tonnages of forages as cash crops on high-value land now being used for crops currently in surplus.

USDA PROGRAM

The major part of the Department's research program on forages is maintained at the Western Utilization Research and Development Division, Albany, California. At the Northern Division, Peoria, Illinois, the Department has a short-term program involving one organic chemist engaged in research to isolate and identify the toxic component(s) of tall fescue grass responsible for a cattle disease known as "fescue foot." This research is cooperative with the Kentucky State Experiment Station which furnishes toxic and nontoxic fescue grass for chemical study and conducts bioassays of fractions and components isolated from fescue at the Northern Division. Liaison is maintained with the fescue breeding program of the Field Crops Research Branch, ARS, through the Agronomy Department of the University of Kentucky and with the Department's Pharmacology Laboratory at Albany, California.

The Federal program at Peoria, Illinois, totals 1.0 professional man-year, all of which is devoted to chemical composition and physical properties.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

Related programs of State Experiment Stations and of industry and other organizations are reported by the Western Utilization Research and Development Division in its "Summary of Current Program and Preliminary Report of Progress."

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Fescue toxicity. The purpose of this research is to isolate and characterize the substance or substances responsible for toxicity of fescue from certain pastures. Fractionation is conducted at the Northern Division, while bioassays of fractions are performed by collaborators at the Kentucky Agricultural Experiment Station. During the past year, the previously reported toxic, water-soluble fraction was further subdivided into (a) alkaloids; (b) other material soluble in chloroform at basic pH; and (c) an aqueous residue. Only the aqueous residue, representing 9.8 percent of the original hay, was found to be toxic to cattle. Kentucky AES is developing a new technique for bioassay by measuring blood circulation in cows' tails. If this new bioassay proves reliable, increased rate of progress will be possible because only 20 percent as much sample as formerly required will be needed. Also, a controlled temperature room should allow assays throughout the year rather than only in cold weather. Kentucky AES has reported that their controlled-temperature enclosure for cattle is nearing completion, and researchers there have requested shipment of fractions for initial testing of the facilities. Three batches of fescue hay were extracted with 80 percent ethanol: (a) 206 pounds of toxic hay; (b) 300 pounds of nontoxic hay; and (c) 300 pounds of presumably toxic hay. The extract was concentrated, and lipids are being removed to give the toxic aqueous extract which will be fractionated and furnished to the Kentucky AES for cattle bioassay. Structure studies on one of the fescue alkaloids indicate a loline-type structure (i.e., pyrrolizidine nucleus with an oxygen bridge).

PUBLICATIONS AND PATENTS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

None.

Line Project Check List -- Reporting Year July 1, 1961 to June 30, 1962

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
N1 1	Corn, wheat, and other cereal crop utilization investigations--Northern region.			
N1 1-58 (Rev.)	Operation and improvement of a culture collection of molds, yeasts, bacteria and actinomycetes to provide a reservoir of authentic microorganisms for use in making antibiotics, vitamins and chemicals, assays, and identifications of importance to national welfare.	Peoria, Ill.	Yes	5-A-1
N1 1-120	Chemical survey of newly introduced or newly developed corn varieties and selections to find high-amylose starch for industrial utilization in fields not open to present commercial starches.*	Peoria, Ill.	Yes	4-A-1
N1 1-126	Stepwise separation of selected constituents of forage grasses such as tall fescue and Italian rye grass that have nutritional or physiological significance, and that are related to the development of the best methods for processing these forages into products of superior feed value.*	Peoria, Ill.	Yes	11-A-1
N1 1-143	New microbial polysaccharides of commercial value produced from cereal grains: Preparation on a semi-pilot-plant scale, purification, characterization, and evaluation of selected products.*	Peoria, Ill.	Yes	5-B-1
N1 1-146	Studies on the fermentative conversion of cereal grain products into carotenes, xanthophylls and related compounds for food, feed, and pharmaceutical use.*	Peoria, Ill.	Yes	5-D-1
N1 1-148	Small-scale engineering studies on wet-milling high-amylose corn to obtain processing data for the production of high-amylose starch and byproducts and to prepare samples for laboratory studies.*	Peoria, Ill.	Yes	4-C-1
N1 1-150	Fundamental and exploratory studies of chemical reactions of dextrose and related carbohydrates in nonaqueous solvents basic to development of new and improved industrial products and chemical raw materials from cereal grains.	Peoria, Ill.	Yes	1-A-1
N1 1-151	Comparative studies on the proteins of corn varieties having starches of widely differing amylose content to provide basic information related to the processing of such new types as high-amylose corn and to the utilization of byproduct protein therefrom.	Peoria, Ill.	Yes	4-A-3
N1 1-152	Investigation of the characteristics and classification of microorganisms of the genus Absidia and its relatives in the family Mucoraceae, with the exception of Rhizopus, as tools for use in the development of fermentations utilizing cereal crops.	Peoria, Ill.	Yes	5-A-4
N1 1-153	Exploration of fleshy fungi, algae and plant cells as fermentative agents for making useful products from cereal grains.	Peoria, Ill.	Yes	5-A-4
N1 1-154	Exploratory studies on the preparation of unsaturated derivatives of starch and dextrins, and their reaction with activated olefinic compounds for conversion of starch into industrial polymers having improved properties.*	Peoria, Ill.	Yes	1-B-3
N1 1-155	Physical and chemical modification of amylose and new high-amylose starches for incorporation into paper products and textiles to provide a basis for expanded industrial utilization of cereal starches.	Peoria, Ill.	Yes	4-B-2
N1 1-156(C)	Evaluation of dialdehyde starch as a tanning agent for sole leather.	Williamsport, Pa. Wyndmoor, Pa.	Yes	1-B-1
N1 1-158(C)	Evaluation of chemically modified high-amylose corn starch (50-60 percent amylose) for use in the paper industry as a basis for the development of expanded markets for cereal grain products.*	Appleton, Wis.	Yes	4-B-3

*Discontinued during reporting year.

Line Project Check List -- Reporting Year July 1, 1961 to June 30, 1962 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
N1 1-159	Development of a process for producing in artificial culture media infective spores for use as pesticidal agents against the Japanese beetle.	Peoria, Ill.	Yes	5-C-1
N1 1-160	Laboratory studies on the transformation of dialdehyde starches to chemical derivatives and low-molecular-weight compounds having potential value for the production of industrial polymers, synthetic resins, plastics, and chemical intermediates.	Peoria, Ill.	Yes	1-B-2
N1 1-161	Investigation of the taxonomic relationships of bacteria in the Pseudomonas fluorescens species-group which are characterized by ability to oxidize glucose in unique manner, thus to facilitate the production of useful substances, particularly fermentation acids, from cereal grain.	Peoria, Ill.	Yes	5-A-2
N1 1-162	Exploratory investigations on the development of principles and procedures for the utilization of wheat and wheat products as constituents of structural products.*	Peoria, Ill.	Yes	2-D-1,2
N1 1-163	Exploratory investigations on enzymatic deamidation of wheat gluten.*	Peoria, Ill.	Yes	5-B-2
N1 1-164	Exploratory investigations on the fermentation production of glutamic acid polymers from wheat gluten.*	Peoria, Ill.	Yes	5-B-2
N1 1-167	Chemical composition and reaction studies on electrophoretically and chromatographically separated protein components found in commercial-type wheat gluten as a basis for increased industrial use of this raw material.*	Peoria, Ill.	Yes	2-A-1,2
N1 1-168	Physicochemical investigations on the molecular properties of electrophoretically and chromatographically separated protein components found in commercial-type wheat gluten, and on their modified forms, as a basis for increased industrial use of this raw material.*	Peoria, Ill.	Yes	2-A-1,2
N1 1-169	Pilot-plant investigations on dry-milling and fractionation of wheat to obtain products having a variety of chemical and physical properties to provide new-type industrial raw materials.*	Peoria, Ill.	Yes	2-C-1,2
N1 1-170	Pilot-plant investigations of conditions and methods for improving tempering and degerminating operations to increase the yield of corn oil and grits by corn dry-milling processes.	Peoria, Ill.	Yes	3-B-1
N1 1-171(C)	Evaluation of cross-linked hypochlorite-oxidized wheat and corn starches in papermaking as a basis for the development of expanded markets for cereal grain products.	Syracuse, N. Y.	Yes	1-B-5
N1 1-172(C)	Investigation of the chemical reactions of periodate-oxidized starch (dialdehyde starch) in solutions involved in its potential practical applications as a basis for improving and enlarging its industrial utility.	St. Paul, Minn.	Yes	1-B-2
N1 1-173(C)	Studies on the preparation of graft copolymers from wheat starch and a variety of non-carbohydrate monomers for conversion of starch into new polymers having properties of value for industrial applications.	Menlo Park, Calif.	Yes	2-B-4
N1 1-174	Screening for microorganisms that can be grown on cereal-based media to produce toxicants, repellents, and attractants, for use as control agents for insects.	Peoria, Ill.	Yes	5-C-2

*Discontinued during reporting year.

Line Project Check List -- Reporting Year July 1, 1961 to June 30, 1962 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
N1 1-175	Investigations on the carotenoid pigments of wet- and dry-milled fractions from corn, including high-amylose types, and of yellow-endosperm sorghum to provide data basic to the most effective utilization of industrial products and fractions from corn and sorghum in feeds.	Peoria, Ill.	Yes	3-A-2
N1 1-176(C)	Polymerization investigations on selected fermentation acids from cereal grains, and on selected fatty acid derivatives from linseed, soybean, and mustard oils, for conversion of these agricultural products to plastics and resins.	Tucson, Ariz.	Yes	6-B-2
N1 1-177(C)	Investigations on the interaction of wheat gluten with dialdehyde starches to improve the adhesive properties of gluten.	Ames, Iowa	Yes	2-B-5
N1 1-178	Investigations on the molecular size and state of aggregation of the amylose and amylopectin components of high-amylose corn starches to provide information basic to industrial utilization.	Peoria, Ill.	Yes	4-A-2
N1 1-179	Basic studies on the chemical structure of the amylose and amylopectin components of high-amylose corn starches to provide information needed for effective industrial utilization of these new starches.	Peoria, Ill.	Yes	4-A-2
N1 1-180(C)	Investigation of factors required by Bacillus popilliae and Bacillus lentimorbus to produce large and vigorous populations of cells in grain-based media for the mass production of spore dusts to control Japanese beetle infestations.	East Lansing, Mich.	Yes	5-C-1
N1 1-181	Studies of the effects of conditioning treatments of wheat on morphological and histochemical characteristics of milled fractions to provide information basic to the production of industrially useful fractions from wheat.	Peoria, Ill.	Yes	2-C-2
N1 1-182	A comprehensive study of sexual agglutination in yeasts as a basis for developing new yeasts and new processes for the fermentative conversion of cereal grains to new products.	Peoria, Ill.	Yes	5-A-3
N1 1-183	Exploratory studies to convert wheat flour into water-resistant, plasticlike chemical derivatives having properties suitable for industrial use in structural and insulating products and in molding compositions.	Peoria, Ill.	Yes	2-B-3
N1 1-184	Chemical conversion of wheat flour into a variety of hydrophilic polymers having a wide range of solubilities and viscosities in aqueous dispersions to meet specific industrial requirements for sizes, adhesives, and thickeners.	Peoria, Ill.	Yes	2-B-1,2
N1 1-185	Engineering development of a fermentation process for the production of citric acid from the wheat starch slurry of the batter process.	Peoria, Ill.	Yes	5-B-3
N1 1-186(C)	Studies on the reaction of acetylene with starch and starch-derived products as a basis for development of new products from cereal grains.	Tucson, Ariz.	Yes	1-A-2
N1 1-187	Isolation and characterization of physiologically active nonprotein nitrogenous substances in corn and corn-milling products as a basis for applied processing studies to increase the use of corn.	Peoria, Ill.	Yes	3-A-1
N1 1-188	Investigations on the preparation of acetal and ketal derivatives of cereal starches to obtain starch products having increased water resistance, flexibility, and enhanced solubility in nonaqueous solvents.	Peoria, Ill.	Yes	1-B-4
N1 1-189(C)	A study of enzyme precursors and the mechanism of enzyme formation during wheat malting to provide basic information needed for the control of enzymes and enzyme action during the milling and processing of wheat.	Manhattan, Kans.	Yes	2-C-3

Line Project Check List -- Reporting Year July 1, 1961 to June 30, 1962 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
N1 1-190(C)	Investigations on methods for the chemical preparation and characterization of amino derivatives of cereal starches by replacement of nonglycosidic hydroxyl groups to obtain new starch products having increased stability to water, dilute acids, and alkali.	Columbus, Ohio	Yes	1-B-4
N1 1-191	Basic taxonomic studies on straight and flexuous streptomycetes of importance to the production of plant antibiotics by fermentation of cereal grains.**	Peoria, Ill.	Yes	5-A-2
N1 1-192	Screening microorganisms that may be grown on cereal-based media to produce antibiotics effective against selected fungal diseases in plants, thus providing new fermentation outlets for cereal grains.**	Peoria, Ill.	Yes	5-C-3
N1 1-193	Investigations on the development of chemical procedures for the production of 5-hydroxymethyl-2-furfural from cereal starch and its derivatives to provide a basis for expanded industrial utilization of cereal starches.**	Peoria, Ill.	Yes	1-B-4
N1 1-194	Search for microorganisms and a fermentative process to convert cereal grain products to xanthophylls that induce desirable pigmentation of poultry products when added to feed.**	Peoria, Ill.	Yes	5-D-1
N1 1-195(C)	Investigations on the alkaline desulfurization of wheat gluten proteins to provide a basis for developing improved modifications of wheat products having utilization potential.**	Lafayette, Ind.	No	
N1 1-196	Chemical investigations on amylomaize selections to guide corn breeders in the development of commercial hybrids containing high-amylose starch for industrial use.**	Peoria, Ill.	Yes	4-A-1
N1 1-197	Engineering process studies on the acid modification of wheat flour to prepare water-dispersible polymeric products and to make quantities available for product evaluation for use as sizes, adhesives, and thickeners.**	Peoria, Ill.	Yes	2-B-1
N1 1-198	Engineering studies on the separation and fractionation of starch from high-amylose corn to prepare a purified corn amylose product for industrial applications and to prepare samples for laboratory studies.**	Peoria, Ill.	Yes	4-B-1
N1 1-199(C)	Investigations on control of the chemical hydrolysis of cereal proteins to provide a basis for development of processes to yield polypeptides suitable for industrial uses.**	Chicago, Ill.	No	
N1 1-200	Evaluation of modified cereal flours and starches as sizing agents, coating adhesives, and wet-end additives for paper in large-scale, high-speed continuous runs.**	Peoria, Ill.	Yes	2-B-1,2,3
N1 1-201(C)	Evaluation of allylated dialdehyde starch as protective and decorative coatings, a molding resin, an adhesive, a modifier for synthetic resins, and as an electrical insulating material for expansion of the industrial use of starch.**	Columbus, Ohio	No	
N1 1-202	Isolation and characterization of the toxic principle in tall fescue responsible for a cattle disease known as "fescue foot" to provide basic information for increased use of this forage.**	Peoria, Ill.	Yes	11-A-1
N1 1-203(C)	Investigations on the control of a selected complex reaction of starch or related carbohydrates through controlled fluid flow dynamics and reaction conditions to provide a basis for process design and improvement leading to increased utilization of cereal grains.**	Baltimore, Md.	No	

**Initiated during reporting year.

Line Project Check List -- Reporting Year July 1, 1961 to June 30, 1962 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
N1 1-204	Chemical investigations on the molecular structure of the protein, glutenin, present in wheat gluten as a basis for increased industrial utilization of this raw material.**	Peoria, Ill.	Yes	2-A-1
N1 1-205(C)	Investigations on reactions of difunctional mercaptans with dextrose, starch, or related carbohydrates to form polymers having potential industrial value.**	Tucson, Ariz.	No	
N1 1-206(C)	Evaluation of beta-carotene product from fermentation of grain-based media with Blakeslea trispora as vitamin A source for poultry and swine.**	East Lansing, Mich.	No	
N1 1-207	Investigations on the enzymatic modification of wheat flour and flour fractions by combined amylases and proteases to provide pastes of suitable viscosities for use as surface sizes and coating agents for paper.**	Peoria, Ill.	Yes	5-B-2
N1 1-208	Investigations on the conversion of cereal grains to economical and efficient soluble fermentation substrates through the action of microbial enzymes, as a basis for increasing the use of these grains by the fermentation industry.**	Peoria, Ill.	Yes	5-B-1
N4 2	Soybean and other oilseed utilization investigations--Northern region.			
N4 2-54	Copolymerization of vinyl ethers of unsaturated fatty alcohols derived from oilseeds with other polymerizable vinyl compounds to give products suitable for use in protective coatings and adhesives.*	Peoria, Ill.	Yes	6-C-1
N4 2-60	Investigations on the cleavage of linseed fatty acids to products of enhanced industrial value: Preparation of new reactive chemicals by oxidative cleavage of linseed fatty acids for use as intermediates for resins, plasticizers, and other industrial products.*	Peoria, Ill.	Yes	7-C-1,2
N4 2-64	Investigations on autoxidation in vegetable oils: Fundamental studies on the reaction of linseed and soybean oils and derived products with atmospheric oxygen to elucidate the mode of oxidation, in order to provide a basis for the discovery of methods of controlling the reaction, and the preparation of new products.	Peoria, Ill.	Yes	7-A-1,2
N4 2-66	Engineering studies on a process for converting soybean and linseed fatty alcohols into their vinyl ethers of which polymers and copolymers have potential industrial uses as protective coatings and adhesives.*	Peoria, Ill.	Yes	6-C-1
N4 2-70	Investigation of methods of converting linseed oil to cyclic and aromatic fatty acids having potential value as new intermediates for industrial chemical products.*	Peoria, Ill.	Yes	6-B-1
N4 2-71(C)	Studies on preparation of copolymers of linseed oil and their evaluation in emulsion paint systems.*	San Francisco, Calif.	Yes	6-C-2
N4 2-72(C)	Basic physical chemical studies on linseed oil emulsions and their interaction with metal oxides.	Los Angeles, Calif.	Yes	6-C-2
N4 2-73	Investigations on the anionic emulsification of commercial linseed oil polymers, or simple modifications thereof, and evaluation of the emulsions as vehicles for paints.	Peoria, Ill.	Yes	6-C-2
N4 2-74	Basic investigations on the selective hydrogenation of linolenic acid as a means of improving the flavor stability of edible soybean oil.	Peoria, Ill.	Yes	7-B-1
N4 2-75(C)	Preparation and evaluation of catalysts for selective hydrogenation of the linolenic acid component of soybean oil glycerides to provide basic information for increased food and industrial applications.	Chicago, Ill.	Yes	7-B-1

*Discontinued during reporting year.
**Initiated during reporting year.

Line Project Check List -- Reporting Year July 1, 1961 to June 30, 1962 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
N4 2-76	Stabilization of solutions and dispersions of soyflour and soybean protein against microbial deterioration for use in industrial emulsions and adhesives.	Peoria, Ill.	Yes	8-C-1
N4 2-77	Engineering studies on the production of cyclic fatty acids from linseed oil.	Peoria, Ill.	Yes	6-B-1
N4 2-78	Chemical and physical characterization studies on the electrophoretically and chromatographically separated proteins found in defatted soybean meal whey, and on their associated nonprotein components to provide information basic to improving the industrial and feed value of whey proteins.	Peoria, Ill.	Yes	8-A-2
N4 2-79	Investigations on the major protein component of the acid-precipitated fraction of soybean proteins to gain basic information for improvement of isolated soybean protein in industrial and food products.	Peoria, Ill.	Yes	8-A-1,B-1
N4 2-80	Engineering studies on a process for converting mustard seed and closely related oilseeds into oil and detoxified meal for industrial feed products.	Peoria, Ill.	Yes	9-B-1
N4 2-81(C)	Preliminary investigations on potential industrial applications for aldehyde oils from soybean, linseed, and mustard oils, their derivatives, and combinations of selected aldehyde oils with cereal products.	Columbus, Ohio	Yes	7-C-2
N4 2-82	Microbial modification of unsaturated fatty acids to produce long-chain amino acids and related valuable derivatives.	Peoria, Ill.	Yes	5-A-5
N4 2-83(C)	Studies on the effect of linseed oil coatings on the durability of air-entrained concrete and its resistance to freeze-thaw cycles for evaluating the use of this oil in protecting this type of concrete against deterioration.**	Manhattan, Kans.	No	
N4 2-84	Exploratory investigations on products obtained by reacting linseed and soybean oils and their fatty acids with unsaturated polyols and polycarboxylic acids, and characterization of the products for utility as water-soluble paint vehicles.**	Peoria, Ill.	No	
N4 2-85	Investigations on the preparation, properties, and reactions of aldehyde oils obtained by the ozonolysis of soybean, linseed and erucic acid oils, as a basis for their increased industrial utilization.**	Peoria, Ill.	Yes	7-C-2
N4 2-86	Investigations on new polymeric products from aldehydic materials obtained by the ozonization of soybean and linseed oils, as a basis for increased industrial utilization of these oils.**	Peoria, Ill.	Yes	7-C-2
N4 2-87	Engineering studies on the production of aldehyde oils from soybean, linseed, and other unsaturated vegetable oils.**	Peoria, Ill.	Yes	7-C-1
N5 1	Sugar and sirups investigations.			
N5 1-69	Investigations on the fermentative production of α-ketoglutaric acid from sugar or molasses to provide new industrial outlets for these agricultural materials.	Peoria, Ill.	Yes	10-A-1
N5 5	New and replacement crops utilization investigations.			
N5 5-15 (Rev.)	Chemical screening to determine the amount and kind of fiber and accompanying constituents in selected plants, as a basis for discovering potential new domestic sources of fiber for pulp and papermaking.	Peoria, Ill.	Yes	9-A-5
N5 5-32	Chemical screening of selected plant seeds to discover sources having vegetable oils in necessary quantity and type to be of potential value for industrial utilization.	Peoria, Ill.	Yes	9-A-1

**Initiated during reporting year.

Line Project Check List -- Reporting Year July 1, 1961 to June 30, 1962 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
N5 5-33	Characterization of selected fractions and chemical components of seeds of plant species containing favorable amounts of gross constituents to obtain more specific evaluation of their potential industrial importance than is afforded by screening analyses.	Peoria, Ill.	Yes	9-A-4,6 9-B-2 9-C-1
N5 5-40(C)	Chemical survey of native, introduced, or newly developed strains of Brassica and related genera of mustard seed to find seed oils with maximum erucic acid content, as a basis for development of new industrial oils from domestic crop sources.	Bozeman, Mont.	Yes	9-A-1
N5 5-41	Investigation of selected plants of the Hibiscus genus, with emphasis on kenaf and okra, to evaluate and develop fibrous products from annual plant sources having superiority or specific preferred properties for industrial use.	Peoria, Ill.	Yes	9-C-2
N5 5-44	Analytical investigations on proteins and other nitrogenous substances in meals from potential new oilseed crops, with emphasis on those bearing oils of high erucic acid content, to obtain fundamental information of value in their processing and utilization.**	Peoria, Ill.	Yes	9-A-2,3
NU P-1	Pioneering Laboratory for Microbiological Chemistry.	Peoria, Ill.	Yes	5-A-5

**Initiated during reporting year.

PL 480 Research Project Check List -- Reporting Year July 1, 1961 to June 30, 1962

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
(10)	Cereal and Forage Crops			
UR-A7-(10)-9	Collection and isolation of molds belonging to the order Mucorales, and classification of the isolates, in order to find microorganisms suitable for fermentative processes of importance in cereal grain utilization.**	Allahabad, India	Yes	5-A-1
UR-A7-(10)-10	A study of survival and possible genetic change in industrially useful microorganisms subjected to lyophilization, to obtain basic information needed for the maintenance of culture collections for industrial fermentation of cereal grains.**	Allahabad, India	No	
UR-A10-(10)-1	A fundamental investigation of the synthesis and chemical and physical properties of multichain polymers and copolymers comprised of amino acids derivable from the cereal grain protein, gliadin and zein, as a contribution to the increased utilization of cereal grains.	Rehovot, Israel	Yes	2,3-A-4
UR-A10-(10)-6	Fundamental studies on the mild oxidation of cereal grain starches by hypochlorite, hypobromite, hypochlorite-bromide mixtures, and other oxidizing agents for the determination of reaction mechanisms and the physical and chemical properties of the modified starches of importance to the production and use of this class of industrial starches.	Jerusalem, Israel	Yes	1-A-3
UR-A10-(10)-9	Studies of the preparation of new cereal starch derivatives by the introduction of fluorine into starch and products derived therefrom, to provide a basis for the increased industrial utilization of wheat, corn, and sorghum.	Jerusalem, Israel	No	
UR-E8-(10)-6	Isolation of organic phosphorus derivatives found in the yeast Torulopsis utilis and elucidation of their structures, to provide new basic information on the fermentation of cereal products to industrial materials by yeasts.	Helsinki, Finland	Yes	5-A-5
UR-E9-(10)-37	Fundamental investigations of the proteolysis-inhibiting effects of cereal flours and starches, and of processing methods for minimizing such effects, to provide a basis for improved quality and increased utilization of cereal products.	Paris, France	Yes	1-A-5
UR-E9-(10)-40	Investigations of the zein protein of corn: Fractionation and study of rheological and physical-chemical properties, chemical composition and structure, and problems of hydration and gelification of fundamental importance to the technology and industrial utilization of corn proteins.**	Paris, France	No	
UR-E9-(10)-42	A fundamental investigation of the physico-chemical alterations brought about in starches and their molecular constituents by gamma-radiations, to provide information needed for modification of starch properties and for the treatment of starch-containing products used industrially or in foods.**	Paris, France	No	

**Activated during reporting year.

PL 480 Research Project Check List -- Reporting Year July 1, 1961 to June 30, 1962 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Sub-Subheading
UR-E15-(10)-21	Investigation of the growth factor (Vitamin B_{13}) of distillers' dried solubles through studies of methods of isolation and purification, mode of formation, and conditions of optimum production by yeast fermentation of cereal grains, to provide basic information for utilizing grains to produce this vitamin.	Milan, Italy	Yes	5-D-2
UR-E15-(10)-24	Investigation of aerobic fermentation processes by measurement of the effects of differences in vessel size and mechanical agitation on the concentration of dissolved oxygen, and by studies of the physiochemical properties of the foam, to obtain fundamental information needed for the increased utilization of cereal grains in fermentative processes.**	Rome, Italy	No	
UR-E15-(10)-25	Investigations of the reaction of cereal starch dextrins with fatty acid chlorides and fatty amines, and evaluation of the products, to provide information important to increasing the utilization of wheat, corn, and sorghum.	Bologna, Italy	Yes	1-B-4
UR-E15-(10)-26	Investigation of the fermentative conversion of glucose to 5-ketogluconic acid through studies of a metabolic pathway in organisms of the Acetobacter genus, to obtain fundamental information for the utilization of grain products in the fermentative production of chemical intermediates.	Milan, Italy	Yes	5-A-5
UR-E15-(10)-32	Investigations on the conformation of glucopyranose rings in amylose corn starches and in linear and cyclic dextrins prepared from these starches, to provide basic information for the chemical modification of starch-derived products for the development of new uses.**	Milan, Italy	No	
UR-E25-(10)-11	Isolation and characterization of yeasts for placement in the Culture Collection of the Agricultural Research Service, as potential agents for the conversion of farm-produced raw materials to products useful to industry and the consuming public.	Madrid, Spain	Yes	5-A-1
UR-E29-(10)-36	Fundamental studies of chemical reactions for polymerizing glucose and glucose derivatives to form new high-molecular-weight compounds, as a basis for the development of new outlets for cereal grains and other starch-rich crops.	Inveresk, Scotland	Yes	1-B-4
UR-E29-(10)-37	Studies on the quantitative measurement of properties of wheat kernels that vary significantly during conditioning, as a basis for improved conditioning of wheat for milling by new and improved methods and increased industrial utilization of flour and milled wheat products.	St. Albans, Herts, England	Yes	2-C-4
UR-E29-(10)-39	A fundamental study of factors governing the onset of oxidative rancidity in oat products, to provide a basis for improving the quality and increasing the utilization of oats in feed and food.	St. Albans, Herts, England	Yes	3-A-3
UR-E29-(10)-40	Investigations of the structure and properties of cereal starches--particularly corn and wheat starches--as revealed by their interaction with enzymes and other proteins, to obtain fundamental information concerning the structure and behavior of cereal starches that would be useful in starch processing.	Edgbaston, Birmingham, England	Yes	1-A-4

**Activated during reporting year.

PL 480 Research Project Check List -- Reporting Year July 1, 1961 to June 30, 1962 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
UR-E29-(10)-51	Investigation of sugars, their phosphate derivatives, and related compounds, as found in molds important to the fermentative conversion of cereal grains to useful products.**	Newcastle-upon-Tyne, England	No	
UR-S3-(10)-11	Preparation of cationic cereal starch derivatives for use in paper and textiles by the introduction of quaternary phosphonium and tertiary sulfonium groups into crosslinked and noncrosslinked starches, to create new markets and expand old markets for starch from cereal grains.**	Rio de Janeiro, Brazil	No	
(40)	Oilseeds			
UR-A10-(40)-17	Fundamental investigations of complexes formed by soybean proteins with other meal constituents, to provide information for applied studies on expanded utilization of soybean oil meal.**	Rehovot, Israel	No	
UR-A10-(40)-18	Investigations of soybean saponins as related to the processing of petroleum ether-extracted meal for feed and to the preparation of soy foods, to provide information basic to improving the nutritional value of soybean protein products.	Rehovot, Israel	Yes	8-D-1
UR-A11-(40)-1(C)	Factory experiments on comparative production of shoyu (soy sauce) from United States and Japanese soybeans, to provide data for the increased use of United States beans.	Tokyo, Japan	Yes	8-B-2
UR-A11-(40)-2	Evaluation of dehulled soybean grits from United States varieties for making miso, to increase soybean utilization in Japan.**	Tokyo, Japan	No	
UR-A11-(40)-5	Investigation of the partial hydrogenation of soybean oil, to produce a stable liquid oil with improved properties for use in Japanese foods.**	Kawagoe, Saitama-ken, Japan	No	
UR-E8-(40)-2	Investigation of continuous multi-stage countercurrent crystallization of linseed and soybean fatty acids as a practical method for producing pure unsaturated fatty acids, to provide a basis for new or improved uses of linseed and soybean oils.	Helsinki, Finland	Yes	7-C-3
UR-E15-(40)-8	An investigation of the minor constituents of linseed oil and their effect on the ability of linseed oil films to spread and adhere to surfaces, as a contribution to the expansion of markets for linseed oil.	Milan, Italy	Yes	6-A-3
UR-E15-(40)-9	Investigations of the controlled thermal polymerization of soybean and linseed oils, and of the separation and characterization of the reaction products, in order to obtain information useful in expanding and improving the industrial applicability of these oils.	Milan, Italy	Yes	6-B-3
UR-E15-(40)-10	Investigations of the effect of metallic catalysts and physical conditions on oxidative cleavage products produced in the autoxidation of polyunsaturated fatty acids, to provide basic information for applied research on the production of new industrial chemicals from soybean and linseed oils.	Milan, Italy	Yes	7-C-4
UR-E15-(40)-14	Studies of the admixture of soybean protein products with wheat flour in the manufacture of pasta (spaghetti, macaroni, etc.) to effect improvements in diets largely based upon cereals and contribute to increased utilization of soybeans.	Rome, Italy	No	

**Activated during reporting year.

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-Subheading
UR-E21-(40)-8	Investigation of the possible role of sterols in the development of flavors and odors in soybean oil through studies of sterol transformations during processing, in order to increase the utilization of soybeans in food.	Gdansk, Poland	Yes	7-A-4
UR-E25-(40)-4	Investigations of ion exchange procedures for removing pro-oxidant metals from soybean oil, in order to contribute to expanded utilization of soybean oil through improvement of its flavor and oxidative stability during transportation, storage, and use.	Seville, Spain	Yes	7-A-3
UR-E25-(40)-29	Improvement of the frying quality of soybean oil through studies of the influence of processing factors and oil modifications on surface tension, viscosity, and other physical properties concerned with its penetration into fried foods, to provide information for increased use in the preparation of Spanish foods.**	Granada, Spain	No	
UR-E29-(40)-29	Development of new uses for soybean and linseed oils through investigations of organometallic derivatives and complexes as components of protective coatings having improved properties.	Teddington, Middlesex, England	Yes	6-C-3
UR-E29-(40)-49	Investigation of the reactions of unsaturated fatty acids and their derivatives in molten alkali, to discover new chemical intermediates important to the increased utilization of soybean and linseed oils.	London, England	Yes	7-C-4
UR-E29-(40)-50	A quantitative study of the polysaccharides in fat-free soybean meal to provide information needed to improve the processing of meal for foods and feeds, thereby contributing to its expanded utilization.	Edinburgh, Scotland	Yes	8-A-3
(50)	Sugar and Miscellaneous Crops			
UR-E15-(50)-29	Preparation and characterization of dextran derivatives, and investigations of their interactions and binding, to provide basic information for increasing the utilization of sugar.**	Rome, Italy	Yes	10-A-2

**Activated during reporting year.